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SURVIVAL OF PATHOGENS IN ANIMAL MANURE DISPOSAL

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SURVIVAL OF PATHOGENS IN ANIMAL MANURE DISPOSAL

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A laboratory model (1:10 scale) of an operational field oxidation ditch used in beef cattle production was utilized in survival and detection studies of Leptospira pomona and Salmonella typhimurium. Minnesota summer (20C) and winter (2C) temperatures, pH, and dissolved oxygen of field ditch manure slurry were simulated in laboratory model studies of manure slurry, effluent, and sludge. Maximum leptospiral survival times of 138 days (summer) and 18 days (winter) in the slurry were measured. Salmonella survival of 47 days in slurry and 87 days in sludge (winter), and 17 days in slurry (summer) were measured. Adequate laboratory cultural detection and isolation techniques were developed to measure survival. Findings from simulated studies in a second laboratory model were used to separate materials for recycling.

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FOREWORD

Man and his environment must be protected from the adverse effects of pesticides, radiation, noise and other forms of pollution, and the unwise management of solid waste. Efforts to protect the environment require a focus that recognizes the interplay between the components of our physical environment--air, water, and land. The National Environmental Research Centers provide this multidisciplinary focus through programs engaged in

- studies on the effects of environmental contaminants on man and the biosphere, and
- a search for ways to prevent contamination and to recycle valuable resources.

In an attempt to solve one of the problems involved in disposing of agricultural solid wastes, a research project was conducted to study the survival of pathogens in animal manure. Because leptospires and salmonella are zoonotic disease pathogens that cause significant problems in the United States, this report will interest persons working not only in solid waste management but in water pollution and public health.

A. W. Breidenbach, Ph.D.
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ABSTRACT

A three year research project entitled the Survival of Pathogens in Animal Manure Disposal was conducted. A laboratory model (1:10 scale) of an operational field oxidation ditch used in beef cattle production was utilized in survival and detection studies of _Leptospira pomona_ and _Salmonella typhimurium_. In the United States leptospires (urinary source) and salmonella (fecal source) are zoonotic disease pathogens which cause significant problems. Minnesota summer (20C) and winter (2C) temperatures, pH and dissolved oxygen of field ditch manure slurry were simulated in laboratory model studies in manure slurry, effluent and sludge. Maximum leptospiral (summer) survival time of 138 days and 18 days (winter) in the slurry were measured. Salmonella survival of 47 days in slurry and 87 days in sludge (winter), and 17 days in slurry (summer) were measured. Adequate laboratory cultural detection and isolation techniques were developed to measure survival.

Simulated studies in a second laboratory model were conducted to define conditions to maintain maximum suspension of solids. These findings were used to separate materials for recycling. Maximum separation of solids occurred when the rotor position was directly above the collection sump.

Because of long term survival of pathogens in the model oxidation ditch and previously documented periods of shedding of weeks to months from infected cattle, a public health problem is created. These findings suggest the need for disinfection of effluents and sludge prior to environmental application for the prevention of disease in man and animals.

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CONCLUSIONS

A laboratory model oxidation ditch of an operational field oxidation ditch (Pasveer) was developed for studying pathogen survival at simulated winter and summer environmental temperature conditions. Methods were developed and utilized to seed, measure survival and detect leptospires and salmonella in an aerated manure environment of the laboratory model ditch and settling chambers. Survival times of *Leptospira pomona* and *Salmonella typhimurium* were measured under summer and winter environmental conditions in beef cattle manure of the model ditch in which temperature, pH and dissolved oxygen were monitored.

Leptospires were measured by artificial cultural techniques to survive at Minnesota summer temperatures (20C) for up to 138 days, when seeded directly in the manure, up to 5 days in effluent and 14 days in sludge of a model settling chamber. At winter temperatures (2C), survival was measured for 18 days when seeded directly in the manure, for 9 days in effluent and 11 days in sludge of the model settling chamber. Since shedding of leptospires in urine of infected animals may occur from 3 to 6 months the problem is increased and these findings are significant. Leptospirosis is widespread in animal populations. Based on laboratory model ditch studies, the urine of infected animals will result in long term sources and vehicles for transmission of leptospires when manure is aerated in an oxidation ditch and subsequently collected in settling chambers as effluent and sludge, prior to discharge to the environment.

Salmonella were measured by cultural techniques, to survive at summer temperatures for 17 days, when seeded in the manure of the laboratory model oxidation ditch, 14 days in the effluent and sludge of a settling chamber. At winter temperatures, survival was measured for 47 days in the manure of the oxidation ditch, 87 days in the sludge and 66 days in effluent of the model settling chamber. In the United States, salmonellosis remains the major zoonotic problem. Animals shedding salmonella in the feces may discharge the bacteria for weeks to months. Based on laboratory model ditch studies the feces of infected animals shedding will result in long term sources and vehicles for transmission of salmonella when manure is aerated in an oxidation ditch and subsequently collected in settling chambers as effluent and sludge prior to discharge to the environment.

The research herein reported, has resulted in a better understanding of leptospirosis and salmonellosis problems as related to the broad fields of agriculture, environment, pollution and public health, as well as to the further knowledge of the specific spirochaete and bacteria in terms of survival.

The variation found in survival times of both leptospires and salmonella may, in part, have resulted from a continuous improvement in laboratory detection and cultural methods of isolating and purifying the pathogens for identification.

Despite qualitative measurement of the ability of these pathogens to survive, their subsequent virulence or the ability to infect warm blooded animals and subsequently man was not determined. The public health effect was determined in that survival of a zoonotic pathogens creates a health problem.

From studies made at the Rosemount Oxidation Ditch it was found that an appreciable portion of some feed rations pass through beef animals and enter the oxidation ditch in undigested form. Such residues are difficult to treat if allowed to remain in the ditch. However, these residues have potential value to the owner for re-feeding (re-cycling) purposes. Hazards of disease transmission introduced when such residues are re-cycled for feed must be defined. The engineering studies conducted in laboratory Model B, provided some data upon which certain design improvements were made in the field, as well as in the laboratory models of the oxidation ditch. Since the principal objective of the study was pathogen survival, major engineering time and effort was directed toward the design, development and maintaining the operation of the laboratory Model A.

Laboratory tests were run in Model B to define those conditions necessary to maintain a condition of maximum suspension of solids within an oxidation ditch. Difficulty was experienced in efforts to design for those conditions necessary to keep all solids in suspension since some particles, being of much greater density than others, required abnormally high velocities in order to remain in suspension. Attempts were made to define conditions which would control, rather than prevent solids settlement. Solids settlement data were used in an effort to determine the feasibility of using the oxidation ditch as a means of separating re-usable solid materials for recycling purposes. Major factors affecting solid settlement were the location of the rotor, rotor immersion depth, and liquid level in the ditch. Regardless of these factors, solids accumulated directly beneath the rotor. Maximum separation of solids from the liquid (87%) occurred when the rotor was positioned directly above the collection sump. Attempts were also made to eliminate reverse flow conditions around each end of the ditch, at which points excessive settlement of solids occurred. Although the temporary insertion of warped sections at strategic locations within the ditch showed some effect in reducing both sedimentation and reversed flow conditions, insufficient data and tests were made to warrant conclusions at this time.

A team effort approach for finding solutions to public health and engineering problems, and those concerned with veterinary medical and environmental problems is essential. This project offered a direct approach to defining present problems and to immediate control and prevention of disease caused by pathogens transmitted by the manure vehicle of domestic animals. The oxidation ditch is essentially a closed system, or may be operated as a closed system. Based on survival of leptospires and salmonella in the sludge and effluents, disinfection appears to be needed prior to environmental discharge. The expanding trend of livestock production by automation and confinement practices presents a unique opportunity for disease control of pathogens originating in animal manures.

RECOMMENDATIONS

Pathogenic microorganisms leptospires and salmonella are capable of surviving in the beef cattle manure of a laboratory model oxidation ditch at Minnesota summer (20C) and winter (2C) environmental temperatures. Since leptospirosis and salmonellosis are zoonotic diseases, they must be considered a public health problem and the feces and/or urine of infected shedding cattle considered a source of these pathogens.

Studies must be conducted to determine if the virulence of these microorganisms continues to exist following survival in the manure of an oxidation ditch.

Disinfection of the effluents and sludge of the extended aeration ditch containing leptospires and salmonella is needed, but to the best of our knowledge a reliable, economical method of disinfection of huge volumes of animal manures does not exist. Further research is essential.

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CONTINUATION STUDY

A continuation study of the Survival of Pathogens in Animal Manure Disposal was approved for funding for two additional years, (1971-1973). The following information briefly indicates the research design, purpose and objectives.

This research was designed to measure and evaluate the public health effect of pathogens, beef cattle manures, extended aeration system of waste disposal and potential pollution of the common environment of man and animals.

1. Determinations will be made of the viability and infectivity of leptospires and salmonellae in aerosols caused by potential mechanical dissemination of these pathogens from manure of a model oxidation ditch. Viability will be measured in artificial culture media and infectivity in laboratory animals.

2. Determinations will be made of the viability and infectivity of leptospires and salmonellae in the feed (corn) recycled from the manure of the field oxidation ditch. Viability will be measured in culture media and infectivity in laboratory animals.

3. Measurements will be made of selected microbial aerosols generated during aerobic treatment of animal manures in an oxidation ditch under a beef confinement housing unit Environmental samplings of aerosols and culturing of fecal-borne bacteria will be made around the field ditch.

4. Relationships between temperature, loading rates and degradation of manure in a model oxidation ditch will be made under controlled environment simulating the field ditch, and further utilized to develop design of the oxidation ditch.

    1. Objectives: The overall objectives are to determine and evaluate:

        a. The public health hazards associated with potential pathogen transmission from the internal and external environment, and from feed recycled from animal manure disposal during aerobic treatment.

        b. The public health hazards created by microbial aerosols in the external environment, disseminated from a field oxidation ditch during aerobic treatment of animal manure.

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c. The relationship between temperature, loading rates, degradation and the effects of change in design in a model oxidation ditch on manure.

INTRODUCTION

Need for Study

Today, quality and ecology of environment is of concern to every segment of society. Environmental pollution has increased the responsibility and inquiry for all persons associated with animal and human health. On August 10, 1970, President Nixon, in presenting the Report of the White House Council on Environmental Quality, stated, "in dealing with the environment, we must learn not to master nature but how to master ourselves,our institutions, and our technology."

While much research has been conducted on handling and treatment of human wastes, and determining the public health significance; very little had been done to define similar problems involving animal wastes. As new methods of animal manure disposal are developed, the public health significance of pathogenic organisms discharged in animal manures needs evaluation. To keep pace with the changing livestock industry practice of expanded confinement and concentration,new methods of manure handling and disposal must be utilized. In 1966 it was reported that 50% of the feedlot cattle fattened in the U.S. were located in 300 feedlot areas (1).

In the United States, increasing centralization of livestock, milk, poultry and egg production has increased disposal and recycling problems associated with more than 1.7 billion tons of animal wastes produced annually. Approximately half of this amount is produced by concentrated systems. As agri-business changes with expansions in animal and human populations, consideration must be given to systematic manure disposal and its public health effect.

Pathogens found in manure are potential contaminants of the sludge when spread as fertilizer on land surface, and of the effluent when discharged on land or into natural waterways; both of these are sources of infection in the environment.

The demand for this study was intended to meet the inquiries growing out of public awareness of the immense problem associated with the effect of animal manures and wastes on environmental quality. In 1965, an Environmental Pollution Panel (2) reported that: "the problem of agricultural waste disposal has grown to such dimensions that probably the major unsolved issue in the confinement housing of livestock and poultry is the handling and disposal of manure." The magnitude of the problem may be visualized in simple terms by comparing the waste voided by man and that by the animals he raises. For example, a cow generates as much manure as 16.4 humans, one hog produces as much manure as 1.9 people, and 7 chickens provide a disposal problem equivalent to that created by one person.

The total volume of animal wastes produced in the U.S. is about 10 times the human population wastes, yet little concern has been given to the former until recent times. In the past, animals were largely produced in unconfined areas where wastes were assimilated by the environment. The livestock industry has rapidly grown from small farm enterprises into great agricultural industry, and the wastes have increased in an unprecedented amount,--for example, the number of beef cattle fattened in feedlots have doubled since 1950--to more than 16,000,000.

The biochemical oxygen demand of wastes of a large midwestern feedlot may be equated to one million people living on 320 acres of land. If these animals are infected, a large number of pathogenic agents may be shed into the environment.

Because of high cost of storing and handling animal manures and their low nutrient value compared to commercial fertilizers, such manures are not always economical for use as soil fertilizers. Today, a large part of animal wastes are recycled to the land as fertilizers by pastured animals depositing manure on the ground. Other common methods are composting, direct spreading on land, lagooning, or spraying.

In the past, economics of agricultural operation has largely determined the management of livestock wastes. In the past with unrestricted development and construction of feedlots, minimal consideration was given to their public health effects or to the resultant interaction of man and lower animals in their common microbe-laden environment. Major changes in waste management methods would be costly for livestock producers. Restrictions are being placed on agriculture. However, guidelines applicable to geographic conditions which protect the livestock producer, the environment, and the health and welfare of the public are essential. To protect public health and reduce disease transmission, animal wastes are being restricted from selected natural waters and land with run-off potential, especially in areas of human population density. Zoning should be considered.

In recent years, local, state and federal governmental agency regulations have developed laws and guidelines, or are in the process of developing laws to minimize the public health hazards of livestock wastes and its subsequent environmental effect. In 1971, the State of Minnesota developed and implemented regulations for control of wastes from livestock feedlots, poultry lots, and other animal lots. In these regulations, standards govern storage, transportation, and disposal of animal wastes, and the registration and issuing of permits for construction and operation of animal waste disposal systems for the protection of the environment.

In order to comply with regulations, and because of economics, new systematic approaches to livestock waste management are being developed and utilized. As modern technology for treating concentrated livestock waste is more commonly utilized, the accompying health hazards to man and lower animals must be evaluated.

Livestock wastes, which include dead animals, meat industry wastes and animal manures, constitute a massive volume of organic and inorganic materials that must be disposed of or recycled.

The secondary treatment of livestock and other organic waste materials involves two biologic processes - anaerobic and aerobic. The anaerobic process involves the use of inorganic compounds, other than oxygen, as the final electron acceptor. Such compounds as used by anaerobic bacteria may be nitrates, sulfates or carbonates. One of the principal advantages of the anaerobic process is the high degree of stabilization which is possible, with carbon dioxide and methane gas as the primary end products. The major disadvantage of the anaerobic systems, as applied to the treatment of livestock wastes, is the high temperature required for optimum operation. The process also requires considerable skill and attention to be assured that proper mixing ratios, pH, and other conditions, are maintained. While many industries and municipalities find it practical to heat anaerobic digestors artifically, such a practice is economically unfeasible for livestock operations. Neither are livestock growers, in general, interested in developing the skills required to operate a good anaerobic digestion system.

The aerobic process,in contrast to anaerobic, utilizes molecular oxygen as the final electron acceptor. Under many natural conditions, such as in turbulent flowing streams, etc., sufficient oxygen is available to satisfy the needs. If the supply and availability of natural oxygen is limited, however mechanical means may be employed to provide additional oxygen. Aerobic bacteria grow rapidly and degrade soluble organic materials very effectively, provided adequate oxygen is available. One principal advantage of the aerobic process as applied to the treatment of livestock wastes is that little or no odor is generated.

Supplemental oxygen may be provided to sustain an aerobic condition in a variety of ways. In some instances shallow ponds or lagoons (about 4 feet deep) may be used. If the loading rate of the waste materials is too great, a deficiency in naturally absorbed oxygen may occur with ponds or lagoons.

Such conditions may require the use of special floating aerators in order to maintain a sufficient supply of oxygen.

Scheltinga (3) and other European investigators, have utilized the Pasveer oxidation ditch as a means of introducing supplemental oxygen into a waste treatment situation. This method is presently in the developmental stage in the U.S. for both municipal and animal manure waste disposal. In 1967 in the United States there were about 400 oxidation ditches in operation; primary agricultural use was in swine operations (4).

The basic operation of an oxidation ditch is similar to that of an aerated pond or lagoon, except that with the former, the liquid waste material is circulated by means of a horizontal-shaft rotor. The purpose of the rotor is (a) to propel the water at a velocity sufficient to keep most solids in suspension and (b) to add oxygen to the waste material.

Recent investigations by Walker (5), Pasveer (6), Morris (7), Irgens (8,9) Dale (10), (11), and Day (12) have shown that the extended aeration process of aerobic digestion of animal manures is practical and has specific advantages over anaerobic digestion methods. The use of the oxidation channel for biologic treatment of animal manures is expanding and appears to be an effective and practical method.

Design criteria used for construction of animal oxidation ditches, and other forms of the extended aeration method, have been based largely on design and data obtained from municipal treatment plants. Differences are noted when one compares human waste to animal waste. Two of the more important differences are: human wastes as collected by the water-carriage system are more diluted with water than are manure wastes from feedlots, dairy or hog barns, or poultry production units; animal wastes contain more slowly degradable materials, such as grain hulls, cellulose and feed fibers.

New systematic approaches to waste disposal are being developed in part in response to society's demand for bettering environmental quality. Human disease problems associated with agricultural occupations have been documented and there is growing recognition and concern of man's contact with livestock wastes through increased recreational and outdoor activities.

More than 150 zoonotic diseases are transmitted between animals and man. Several hundred diseases are transmitted from animal to animal. Many of the etiologic agents are shed in animal wastes, or contaminate animal wastes where adequate nutrients for survival and growth may be found. At times agricultural and recreational use of lands may conflict ; with combined usage of surface waters from the health point of view.

Wedum and associates (13) reported on a survey of recovery of specific microorganisms from urine and feces of experimentally infected cattle and found that agents of 14 specific disease entities were recovered from the feces of cattle infected with adenovirus, anthrax, brucellosis, Coxsackie virus A, Coxsackie virus B, enterovirus, foot and mouth disease, leptospirosis, psittacosis - ornithosis, Q-fever, reovirus, rinderpest, tuberculosis and tularemia; and 7 agents from urine of experimentally infected cattle, (brucellosis, foot and mouth, leptospirosis, Q-fever, rinderpest, tuberculosis, and tularemia). This report excluded most intestinal diseases.

Epidemiologic investigations have associated pathogenic microorganisms of animal waste origin with outbreaks of human disease. Decker and Steele (14) report that human health problems are created by bacterial zoonoses. These include leptospirosis, salmonellosis, staphylococcal and streptococcal infections, tetanus, brucellosis, tuberculosis and colibacillosis and diseases by other classes of pathogenic agents which occured following contact with wastes. Animal wastes also serve as breeding grounds for many vectors essential for viral transmission.

In an extensive literature survey titled "Solid Waste/Disease Relationships," Hanks (15) states that the literature fails to supply data which would permit a quantitative estimate of relationship between solid waste and disease. He further states that circumstantial and epidemiologic information presented in reports does support a definite relationship of disease and solid waste --including animal waste. He further states that in developed countries reported incidence of human infections traceable to animal fecal wastes is low--but suspicion is that the actual number of cases is probably much higher.

In implicating livestock wastes as vehicles of disease, many variables affecting the host-agent-environment relationship exist under field conditions.

A decision was made to study the pathogenic agents of two disease entities that effect both animals and man. The leptospiral and salmonella organisms were chosen because they are shed, respectively, in urine and feces of infected animals.

It is anticipated that in the future many farmers in Minnesota and other parts of the United States will construct oxidation channel facilities similar to the field unit now in operation at the Rosemount Agricultural Experiment Station of the University of Minnesota. The following objectives were developed.

Major Objectives were on the:

1. Survival of Leptospira pomona and Salmonella typhimurium in cattle manure disposal under specific, controlled environmental and physical conditions.

2. Comparision of methods for qualitatively determining the survival of these two pathogens (L.pomona and S. typhimurium) in animal manure.

3. Simulation, production and maintenance of field environmental conditions in laboratory model research units.

4. Establishment of criteria in the hydraulic and structural design of oxidation channels, vertical aerators, other forms of extended aeration devices; permissible loading rates of solid waste into aeration devices, especially at warm and cold temperatures, and the effect on survival of pathogens.

The more specific aims were:

1. Determining and evaluating potential public health hazards created by the extended aeration process, if survival of Leptospira pomona and Salmonella typhimurium occur in either the effluent or sludge of operational laboratory models.

2. Determining the effect of specific field environmental conditions and chlorination upon the survival of L. pomona and S. typhimurium and the comparision of methods for detection of pathogens in laboratory models.

3. Determining under laboratory-controlled conditions: the performance of oxidation channel models when loaded with beef animal manure.

Organization for Study

The overall project direction was provided by principal investigator Dr. S.L. Diesch, Department of Microbiology and Public Health, College of Veterinary Medicine. He was assisted by co-principal investigator Dr. B.S. Pomeroy, Professor and Head of the Department of Veterinary Microbiology and Public Health.

For many years Dr. Pomeroy has been active in the prevention and control of salmonellosis. During the final year of the study, Dr. L. Will, Research Associate, Department of Veterinary Microbiology and Public Health, contributed greatly to the microbiologic aspects, the total research efforts and to the preparation of this final project report.

The microbiologic studies of the leptospires and salmonella survival and detection were conducted in the laboratories of the Department of Veterinary Microbiology and Public Health. Most of these studies were conducted in a laboratory model designed and developed to simulate the field oxidation ditch.

Two laboratory models were designed, constructed and maintained by co-principal investigator Professor E.R. Allred of the Department of Agricultural Engineering, University of Minnesota. Professor Allred directed and conducted the engineering aspects of this research in the laboratories of the Department of Agricultural Engineering. Total solids determinations of the liquid manure from the field and laboratory ditch model were conducted in the Agricultural Engineering laboratories. Mrs. Jenny Trombley and Mr. Egon Straumann were employed for the total of the three year project and were invaluable laboratory technical and scientific personnel. On a part-time basis veterinary medical and engineering students were employed throughout the study.

An essential part of this research project was the prior development, construction and utilization of the field unit oxidation ditch which had been operational at the Rosemount Abricultural Experiment Station since 1967. Beef cattle were housed on slatted floors and fattened over the ditch, thereby setting-up a realistic field situation. Credit for the development and maintenance of the field operational unit goes to members of the Departments of Agricultural Engineering and Animal Science. Financial support for the field project was obtained from the University of Minnesota Agricultural Experiment Station and the U.S. Department of Agriculture. Essential environmental data to determine summer and winter conditions were gathered from the field ditch unit. Samples of liquid manure were collected from the field ditch unit and utilized for survival studies in the laboratory model ditch of the Department of Veterinary Microbiology and Public Health.

Nature and Scope of Study

Efforts were made to develop new methods and further refine standard methods of pathogenic leptospira and salmonella detection. The measurements of maximum viability of these pathogens were qualitative determinations.

Another important aspect of this study was the design, development and utilization of laboratory models to simulate, not duplicate, environmental field conditions. It was possible to seed a laboratory model ditch with pathogenic bacteria. Seeding a field ditch with pathogens would have been undesirable.

We had initially proposed to collect and study effluents discharged from the field operation ditch, however, during the three years of study no effluents were discharged. Effluents for research utilization were developed for pathogen survival and detection studies by allowing liquid manure from the laboratory ditch model to separate by gravitation into effluent and sludge.

Laboratory units were utilized as models to study the survival of pathogens and the engineering aspects under simulated conditions of the field units to determine the public health significance. Studies were conducted to determine pathogen survival at specific pH, dissolved oxygen, temperature and total solids levels. From findings during the operation of the field facility at Rosemount and from other studies, it was apparent that because of differences, it was difficult to employ the same design criteria for human wastes as for animal wastes in the construction, operation of oxidation channels, and the subsequent effect on pathogen survival. The most difficult problems arose because of differences in: a) total solids present in waste liquors; b) rate of settlement and stablization of solids; and c) physical effects of large solid particles on hydraulic flow patterns within an oxidation ditch. There was evidence that the hydraulic efficiency of the oxidation channel must be improved in order to avoid settling-out of solids in certain areas, with subsequent creation of odorous and anaerobic conditions. Laboratory trials by Dale (8), and field ditch observations by Day (10) and Allred (Co-principal investigator) indicate that screening of undigested materials is desirable before the animal wastes reach the ditch. Current investigators have generally found the need for a holding basin (lagoon, pit, settling tank) to permit flocculation and settlement of solids. Studies to determine the survival of pathogens and the effect on the health of man and animals had not been evaluated. Research was conducted to determine if the extended operation process has a lethal effect, or no effect, or promotes viability of pathogens. It was proposed that research could best and most economically be conducted under laboratory controlled conditions.

Future systems developed and utilized for animal manure disposal must encompass consideration for all aspects of public health as a means of prevention and control of disease in both man and animals.

Methods of Procedure

Field Oxidation Ditch Facility

During recent years agricultural engineers at the University of Minnesota and the U.S. Department of Agriculture have conducted experiments on treatment of beef cattle wastes in an oxidation ditch. The function of the oxidation ditch is to promote aerobic degradation of the animal manure. A liquid medium is provided within the ditch to collect and treat the animal waste as it enters. This treatment process varies, depending on the objectives and the particular system involved. One system provides partial treatment in the oxidation ditch and relies on an external lagoon to provide additional treatment until final disposal or utilization occurs. A batch type oxidation ditch may be used to provide containment and partial treatment until the waste can be removed and disposed of on the land for final treatment.

The oxidation ditch provides several advantages to the beef operator.

1. One of the primary concerns is odor control. A rotor provides aeration and controls velocity of the flow. In doing so, an aerobic condition is maintained and subsequent degradation or breakdown of the waste is odor free.

2. The second advantage offered is that of storage. Storage reduces the possibility of runoff and pollution of surface and groundwaters, since the operator may dispose or utilize the animal waste on agricultural land when and where the soil can accept the waste. If this is done sometime in late spring through early fall, maximum utilization of the nutrients and organic matter in the waste is obtained. Spreading through the summer may also result in runoff under certain conditions (in Minnesota).

3. The treatment received by the waste in oxidation ditches reduces the pollutional strength of the material and in so doing reduces the potential hazard which may occur from land spreading. The levels of chemical and biochemical oxygen demand, and solids can also be reduced in an oxidation ditch.

4. The use of slatted floors above an oxidation ditch also results in a reduced labor input into the waste collection treatment process.

The objective of the above research has been to explore the use of the oxidation ditch for the handling of beef cattle waste by providing a storage-treatment system with maximum odor control and minimum labor requirements. Figure 1 shows a floor plan of the pilot scale oxidation ditch as it appears at the Rosemount Experiment Station, Rosemount, Minnesota. It is a race-track type of oxidation

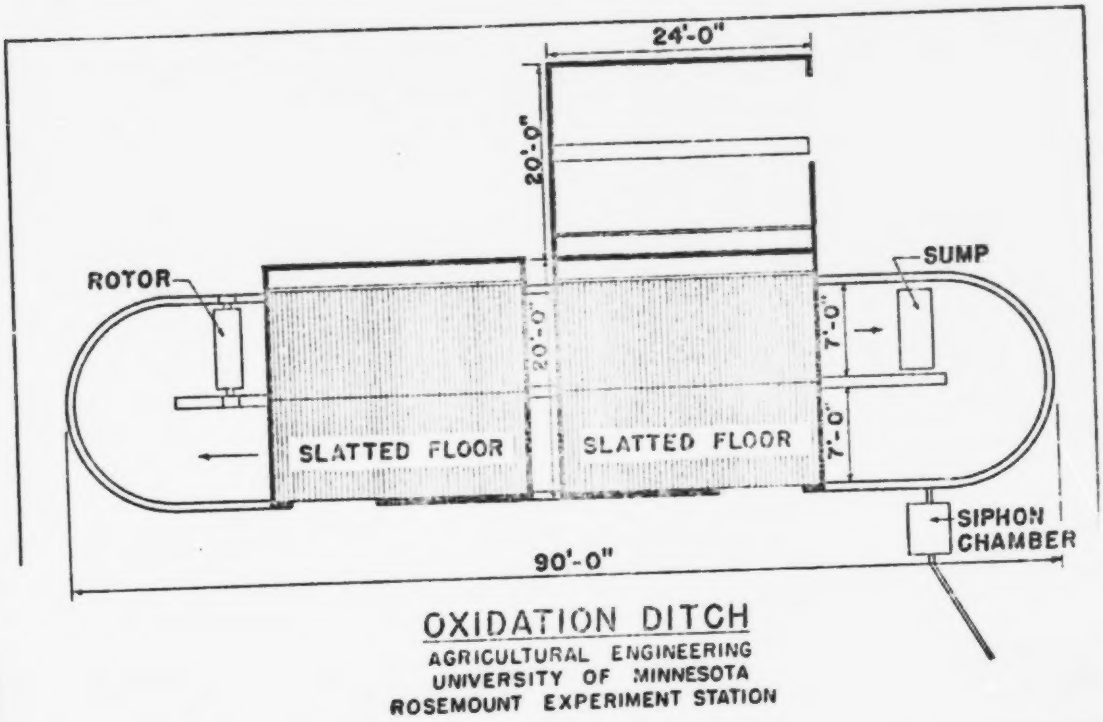
24'-0"
20'-0"
ROTOR
SUMP
20'-0"
7'-0"
7'-0"
SLATTED FLOOR
SLATTED FLOOR
SIPHON CHAMBER
90'-0"
OXIDATION DITCH
AGRICULTURAL ENGINEERING
UNIVERSITY OF MINNESOTA
ROSEMOUNT EXPERIMENT STATION

Figure 1 Plan view of the oxidation ditch and research structures at Rosemount, Minnesota.

ditch which provides a continuous channel 172 feet long, 7 feet wide, and 4½ feet deep. Poured-in-place concrete was used to eliminate any losses to percolation. The ditch is located within a rigid,framed, steel building enclosed on three sides. Within this building are three beef-feeding, environmental units constructed to evaluate the effects of the system on the management and feeding of beef cattle. Two of these units are located over the oxidation ditch and the waste generated passes through the slots to the liquid below.

An initial group of 60 Holstein steers was placed in the field unit on 11/4/67. Water was added to the ditch and the rotor started in January, 1968. A second herd, of 45 Holstein steers was placed over the unit on 6/6/68 and removed on 10/1/68. The liquid ditch temperature ranged from 16 degrees to 20 degrees C. From this herd of cattle the liquid manure samples were utilized in the laboratory of this research project. After each herd was sold to slaughter most of the sludge was removed and spread on the fields, and the remaining portion used for seeding purposes in the next batch.

A third herd of 36 Hereford cattle was placed in the field unit on 11/5/68. The liquid ditch manure temperature ranged from 2.6 degrees to 0.2 degrees C. for November, December and January. During this period, severe foaming of the ditch was encountered which may predispose to aerosol transmission of pathogenic organisms. The pH ranged from 8.2 - 8.3 during this same period. The dissolved oxygen (D.O.) was 0.5 - 1 ppm of the liquid manure and sludge were determined. This information has been utilized in the laboratory model studies.

Tests of the treatment and storage of beef waste in the oxidation ditch at Rosemount were begun in December, 1967. Starting loading rates for four experiments have been 210, 138, 38, and 50 cubic feet of water per animal, respectively. These experiments were conducted on a batch basis.

Continous measurements of dissolved oxygen, BOD, nitrogen, and solids were made to define the functional operation of the Rosemount Oxidation Ditch. No attempt was made, however, to determine the extent of the survival of disease pathogens in the Rosemount or other ditches. Construction and operation of the Rosemount facility was financially supported by the University of Minnesota Agricultural Experiment Station and the U.S. Department of Agriculture, (16, 17, 18, 19, 20, 21, 22).

The operational Rosemount Field unit of the oxidation ditch was essential to this research project as it provided experimental parameters which were utilized in both laboratory models (Figures 2,3). The data of environmental conditions from the field oxidation ditch was utilized in the laboratory studies.

Development of Scale Laboratory Model Oxidation Ditches

Two laboratory models (1:10 scale) of the field oxidation ditch at Rosemount were constructed. To simulate winter liquid temperature conditions, as observed in the field ditch, it was necessary to insulate the models by wrapping with two inch-thick rigid styrofoam. In order to provide portability to the models, the original cooling system was completely redesigned. A three-quarter horsepower cooler-condenser unit, installed directly beneath the ditch, served to cool the ethylene glycol solution, which was pumped and circulated through the stainless steel trough dividing wall within the ditch. An overflow of coolant into the liquid manure caused termination of an experiment on leptospiral survival and the loss of several weeks research time. It was again necessary to redesign the cooling system into a closed system of coils centered in the ditch. Since the models were operated in indoor heated laboratories, the cooling system operated much of the time. Provision for slight warming of ditch liquids has also been provided and were used during survival studies under summer (warm) conditions.

The final ditch design also included the addition of a plexiglass cover. This cover provided the operating personnel protection against aerosols which may contain pathogenic microorganisms. It also provided greater uniform environmental conditions at the liquid-atmosphere interface. Work in improved engineering design criteria of the laboratory oxidation ditches and its components were accomplished during the construction phase of the laboratory models.

Utilization of .the Model A Oxidation Ditch

In the first model (hereafter referred to as Model A) efforts were made to simulate environmental conditions existing at the Rosemount field oxidation ditch. (Figure 4). The following seasonal data was collected in 1967-68 from the operational field ditch over which 36 head of beef cattle were housed: pH ranged from 6.8 - 8.4; winter ditch temperatures ranged from 1.7 - 6.4C and summer from 13 - 25C. Total solids ranged from 5,802 - 135,333 mg/liter. Since the field ditch was tested experimentally for maximum loading capacity, no effort was made to simulate the total solids levels of the field

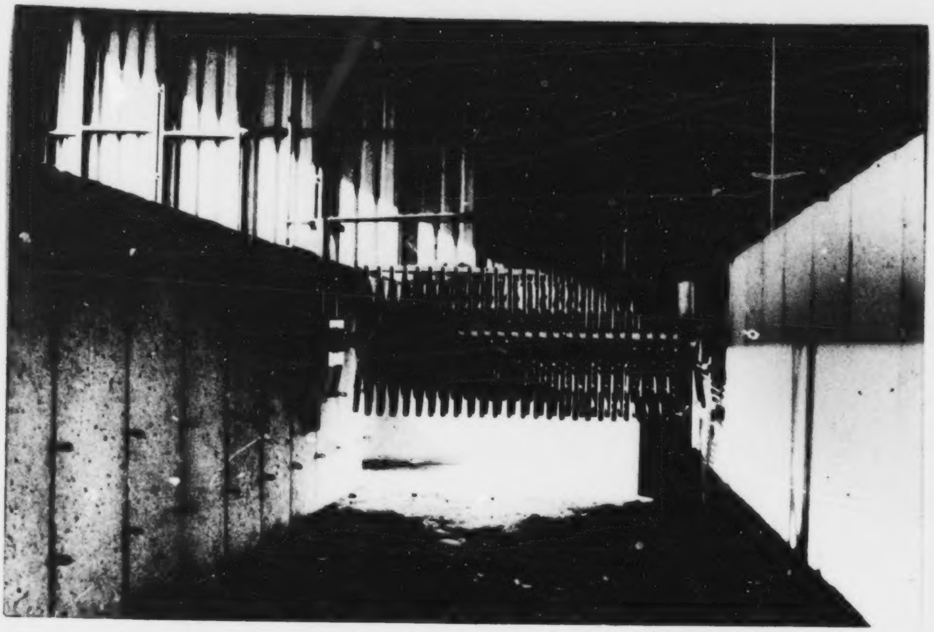

Figure 2. Field oxidation ditch, rotor. Rosemount Experiment Station (University of Minnesota).

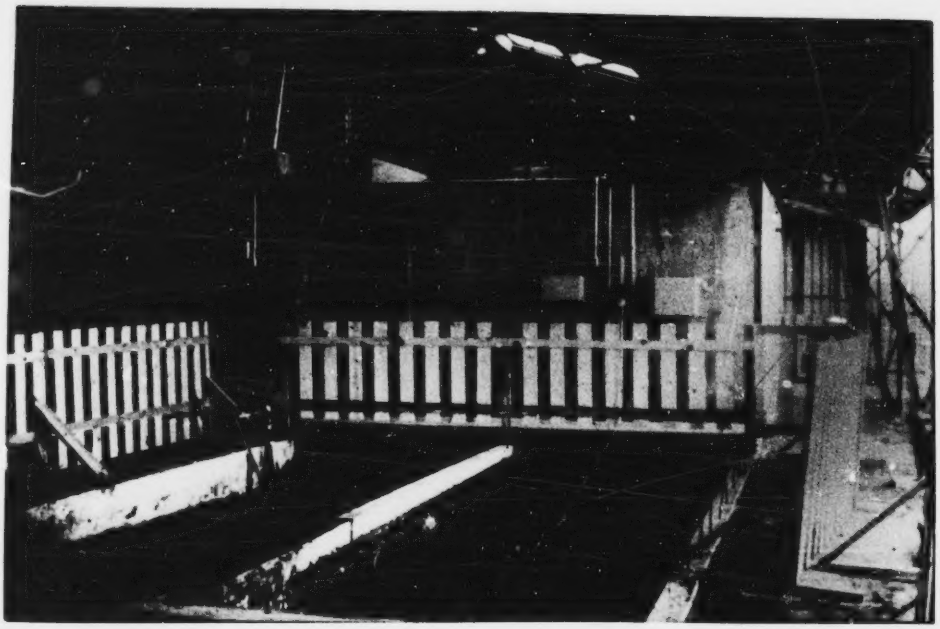

Figure 3. Cattle unit housing 18 steers above an operational oxidation ditch. Rosemount Experiment Station,(University of Minnesota).

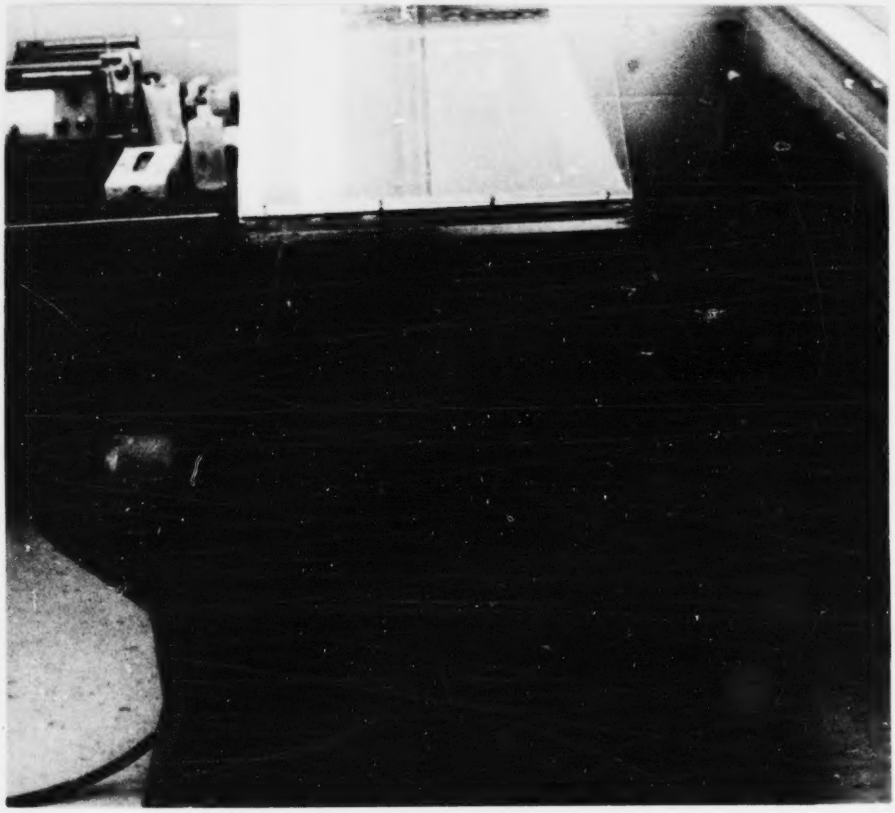

Figure 4 Laboratory Model A, a 1:10 scale model of an operational field oxidation ditch, contains beef cattle manure. Rotor is shown on the left.

ditch in Model A. Attempts were made to maintain the laboratory model total solids at levels from 5,000 - 10,000 mg/liter. This range of total solids had been reported as being maintained in oxidation ditches used for human sewage disposal. Attempts were made to maintain the dissolved oxygen (D.O.) between 1 and 5 ppm in the liquid manure of the laboratory model.

Model A was filled with 113 liters of manure (liquid media) from the field ditch. Following addition of manure the laboratory model oxidation ditch was allowed to function a minimum of 1 week to stabilize the environmental conditions. At least once a week fresh samples of liquid manure were transported in 5 gallon jugs from the Rosemount field ditch to the laboratory. The manure was refrigerated at 2C until added to the laboratory model. Each lot was sampled and examined for presence of leptospires by darkfield microscopy and fluorescent antibody technique. Isolation attempts were made in artificial culture media. Lots were examined for salmonellae by the fluorescent antibody technique and cultural procedure. Only lots found negative were added to the ditch. During initial experiments 2.2 lbs. of liquid manure was added each day to the ditch. Due to a build up of total solids (above 10,000 mg/liter) daily additions of manure to the ditch were discontinued. Additions were made intermittantly to maintain the total solids at 10,000 mg/liter. It was occasionally necessary to add unchlorinated well water from the Rosemount station well to maintain desired operating range of total solids.

Portable effluent chambers,as settling chambers,were designed and constructed by the agricultural engineers to hold 1,000 ml. of liquid manure. The material used was plexiglass. Initially, the environmental temperature was maintained by placing the chamber in the liquid manure of the ditch (Figure 4). Subsequent studies were conducted with the chamber removed from the laboratory model ditch. During summer temperature studies, foaming of the liquid manure in the laboratory ditch was a problem. This foaming occurred during laboratory ditch start-ups. The foaming was reduced by slowing the rotor speed and spraying the foam with Dow-Corning Anti-foam A.* As the ditch stabilized, the problem appeared to cease. During this same time period, foaming was a problem in the field ditch operation, following removal of total solids and re-stabilizing the ditch.

Temperature, pH and D.O. were monitored and recorded at 6 hour intervals by use of monitoring probes, (Figure 5). To maintain uniform external environmental conditions, room

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*Dow Corning Antifoam A Spray
Dow Corning Corporation
Greensboro, North Carolina

Figure 5 Laboratory model A oxidation ditch with monitoring probes shown on left used to collect pH, D.O. and temperature data. Selas candles shown on right were used to contain leptospires during one phase of survival studies.

temperature, barometric pressure and per cent relative humidity were recorded daily.

Utilization of Model B Oxidation Ditch

The second oxidation ditch model, illustrated in Figures 6 and 7 and hereafter referred to as Model B, is a duplicate of Model A except that a sump or storage pit was installed in the former to facilitate the separation and removal of specific solid materials. Two separate rotor designs were also constructed and tested in Model B. Early tests were made using a brush-type rotor but its use was discontinued when it became obvious that insufficient water velocities were being generated. Studies of the engineering aspects of oxidation ditch operation were conducted in the Agricultural Engineering Waste Research Laboratory, under the direction of Professors Evan R. Allred, Co-principal Investigator, Phillip Goodrich, and James A. Moore, all of the Department of Agriculture Engineering.

Investigations involving engineering design and operation of the oxidation ditch focused upon the following major objectives:

1. Determination of the effect of the location, speed and immersion depth of the rotor upon solid settlement patterns.
2. To observe and measure the movement of solids for varying stream-bed configurations and water depths.
3. Determination of the effect of rotor immersion and water depths on velocity distribution patterns within the ditch.
4. Establishing criteria for design of field rotor units.

Figure 6 Model B oxidation ditch in which velocity distribution and solid sedimentation studies were made.

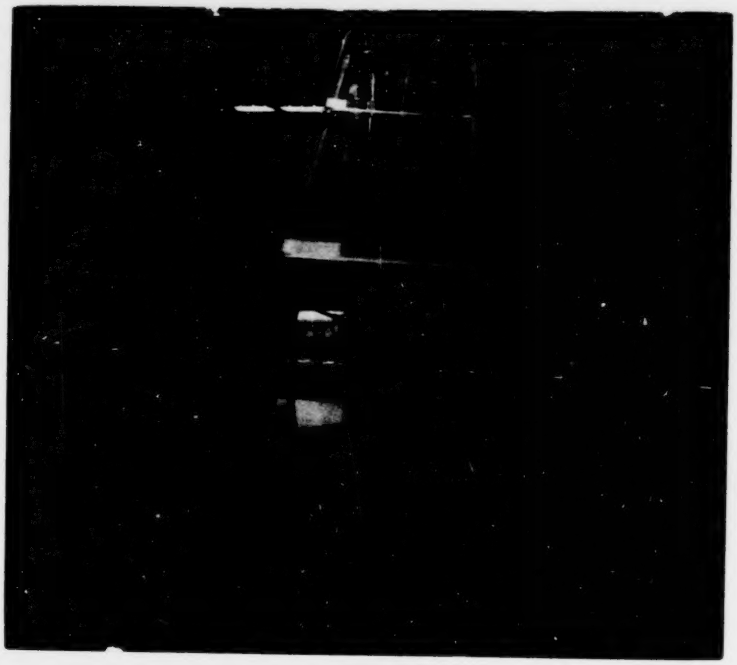

Figure 7 Overhead view of Model B oxidation ditch.

SURVIVAL AND DETECTION OF LEPTOSPIRES IN BEEF CATTLE MANURE

Introduction

Leptospires are pathogenic microorganisms which are mainly shed in the urine of infected animals. The disease is widespread in cattle, swine and many other animals. Large numbers of leptospires (100,000,000 per ml) in the urine have been reported (23). Infected cattle are frequently shedders of the organisms for periods up to several months. The agent lives for extended periods of time in the environment and is transmissible to both man and animals.

Leptospirosis has been referred to as the world's most widespread, contemporary zoonosis. Although only 52 human cases of leptospirosis were reported in the U.S. in 1970 (24) there is evidence that many cases are not diagnosed and reported (25,26). In the United States several outbreaks of human leptospirosis have been associated with *L. pomona* infection from contact with water contaminated with urine from infected cattle (25,27). In the U.S., serotype *pomona* is most commonly reported in cattle. One outbreak described 40 human cases in Iowa. In Iowa in 1963, Diesch and McCulloch (28) reported 15 cases following swimming in a farm creek and *L. pomona* was isolated from the swimming site. Forty cases were reported to have occurred among packing house workers in Iowa (29). Sixty-one human cases occurred in Washington following swimming in irrigation ditch water contaminated by urine of infected cattle pastured nearby (30).

According to Gillespie et. al. (31), Chang, Buckingham and Taylor (32), and Ryu and Liu (33), leptospires may live in water for several weeks. The overall public health effect and impact of this disease has been well documented.

From 1951-1960 (34) the estimated average annual loss to the livestock industry due to leptospirosis (dairy and milk) was $12,189,000. The Leptospirosis Committee of the United States Livestock Sanitary Association (35) stated that the disease was not amenable to eradication and cited the need for continuing prophylactic programs in the problem herds to provide protection against cyclic recurrence of infection.

Materials and Methods:

Development

The *Leptospira pomona* MLS strain originally isolated from infected cattle, was selected as the serotype to be utilized in the survival studies. In preliminary studies, intraperitoneal inoculation of leptospires into weanling hamsters caused death. Known cultural methods for maintaining and growing adequate numbers and inoculating known numbers into the laboratory model oxidation ditch were utilized, and newer and improved methods were continuously evaluated.

Initially, there was a definite need for adequate laboratory methods to measure survival of leptospires in animal manures under specified field environmental conditions. Under standard laboratory procedures it was difficult to obtain contaminant free cultures. Developing satisfactory methods for selective isolation of leptospires from massivly contaminated media, such as beef cattle manure, posed a difficult problem.

Developing methods for containing leptospires in a specific area of the model ditch was a major problem. This had to be resolved by developing a suitable chamber to retain the leptospires suspended in the manure medium. The chamber or container had to contain an adequate number of leptospires for repeated sampling and allow nutrient exchange and maintain comparable environmental conditions as found in the laboratory ditch manure. Several preliminary studies were conducted using colloidal sacks, mylar sheeting, millipore filters and Rose perfusion chambers, none of which were found to be suitable for this study. The cellulose base filters were digested by cellulose digesting organisms found in the beef cattle manures and subsequently the leptospires escaped.

Methods were developed to wash and concentrate leptospires for inoculation into the laboratory oxidation ditch and the isolation chamber. The organism is fragile and care must be used in this procedure not to cause fragmentation or injury to the leptospires.

The procedure for isolation of leptospires from the laboratory model oxidation ditch and subsequent identification, to ascertain survival times, was developed. These methods are described in the text. In an attempt to develop new and more accurate methods, alternative methods were constantly considered and tested throughout the three year study. The solids and the viscosity of the mixed liquor of the oxidation ditch posed problems in attempted pre-filtering prior to isolation and identification. Various methods of isolation by filtering, centrifugation and gradient centrifugation, serial dilution and agar plating techniques were tested as they were developed.

Growth of Leptospiral Inoculum

Leptospires for inoculation (seeding the laboratory model ditch) were propagated in bovine serum albumin culture medium (36). To inhibit contaminating bacteria, 5-fluorouracil (100 mcg/ml) was added to the media (37). Actively growing

5-7 day cultures were centrifuged at 10,000 rpm for 5 minutes, the supernate drawn off, and leptospires resuspended in phosphate buffered saline.

Numbers of concentrated leptospires were estimated by utilization of the Coleman Model 9, Nephlo Colorimeter, and readings from a standardized curve with Nephlos units against the numbers of leptospires per ml. of suspension. (Figure A, Appendix A). The actual count of leptospires was determined with a Petroff Hauser Counter.

Methods of Seeding for Survival Studies

1) Seeding in Selas Porcelain Candles Suspended in Laboratory Model A Ditch.

Extensive research was conducted on the use of an isolet chamber utilizing Gelman glass filters attached to the ends of a circular pipe for containing leptospires. Because of contaminant overgrowth within the chambers the use of the isolet unit was discontinued. Following experimental evaluation of many other methods, Selas porcelain candles, 0.3 micron porosity, were utilized to contain leptospires and allow nutrient exchange with the liquid manure environment. Three Selas candles were used in each experiment indicated, and suspended in the manure of Model A (Figure 5). The model oxidation ditch cover shielded the open end of the candles from the external environment.

Candle A contained phosphate buffer solution and leptospires (control).
Candle B contained sterile (autoclaved) manure and leptospires (control).
Candle C contained manure and leptospires.

Three hundred million leptospires (3cc of 25 Nepholos BSA culture) were placed in the material of each candle. During each experiment conducted, the contents of the candles were sampled daily and examined by Darkfield microscopy to detect leptospires. The pH, D.O. and temperature were monitored from the ditch environment.

2) Seeding in Liquid Manure of Laboratory Model A Ditch.

It was postulated that 36 beef cattle housed over the field ditch may shed leptospires in the urine at a maximum rate of 100 million per ml. if infected. The leptospiral concentration used for seeding amounted to a concentration of 1:1000 ratio, and potential leptospires in urine (37.5 billion) were added daily for 5 days.

3) Seeding in Effluent and Sludge of Model Settling Chamber

The settling chamber was developed as a model of that designed for use in the field oxidation ditch. Liquid manure (1,000 ml) collected from the surface (top 1") of Model A was placed in the settling chamber. In several hours, the liquid manure separated into distinct zones of effluent and sludge. Approximately 1.2 billion leptospires were added to the chamber. The environmental temperature of the settling chamber was maintained at summer and winter conditions.

Maintenance of Study Environment in Laboratory Model Oxidation Ditch

1) Total Solids

Initially, attempts were made to add approximately 2.2 lbs. of liquid manure (collected from the field oxidation ditch) each day to the ditch. This amount simulated the ratio of defecation and urination of cattle housed on the slatted floor above the field ditch. This daily addition was discontinued and an intermittant addition of liquid manure was utilized to maintain the total solids at 5,000 - 10,000 mgs/l. When total solids were found higher than 10,000 mgs./l it was necessary to add unchlorinated Rosemount well water to lower the total solids.

2) pH

In most instances the pH range of the manure stabilized to simulate the range of the field ditch (Appendix A). Daily additions of small amounts of manure lowered the alkaline pH.

3) Temperature

The ambient temperature was maintained in the range of 2 - 5C (winter) by utilization of the refrigeration unit and insulation of the laboratory model ditch. It was also necessary to alter and stabilize the room temperature to effect maintaining summer ambient temperature, 20C was more difficult to maintain. The situation was improved by installation of a window air-conditioner to maintain a more stable room temperature.

4) Dissolved oxygen (D.O.)

During summer temperature studies, the D.O. of the liquid manure was maintained at 5 ppm by regulating the speed of the rotor. No difficulty was encountered in maintaining these levels in summer studies.

During winter environmental studies (2 - 5C) it was nearly impossible to maintain the D.O. at 5 ppm. When the rotor was stopped the D.O. reading was higher than 5 ppm. Addition of small amounts of manure lowered the D.O. level reading.

Methods of Sampling for Leptospires

1) Selas Candles

Selas porcelain candles (No. 03 which are 0.3 porosity) were utilized. Samples for detection and measuring survival were collected from the candles by use of a disposable pipette with an attached bulb.

2) Laboratory Model Ditch Manure

Daily collection of manure samples were made by pipette from the top, middle and bottom of the liquid manure in the laboratory model ditch. Two collection sites were (a) in front rotor, where material was thoroughly mixed, and (b) other side of divider, where settling of solids occurred.

3) Settling Chambers (Effluent and Sludge).

Samples were collected by pipettes at the top, middle and bottom of the effluent settling chambers. The top and middle zones were relatively clear and considered effluent. The bottom was solids and considered sludge.

Methods of Detecting Leptospires

1) Fluorescent Antibody Studies (FA) to Detect Leptospires

After testing of several fluorescent antibody methods the following procedure was developed to detect leptospires. However, the FA technique does not measure survival.

For description of method used for fluorescent antibody staining see Appendix A.

2) Darkfield microscopy.

All manure samples and inoculated culture media were examined (645 x magnification) for presence or absence of leptospires.

Methods of Measuring Leptospiral Survival

(1) Tube Dilution Procedure for isolating leptospires

Tubes containing (8 ml.) liquid culture medium (bovine serum albumin) were inoculated daily with specimens from the manure seeded with leptospires. A 10-fold serial dilution procedure was utilized. The tubes were incubated for growth of leptospires at 30C and examined microscopically at 7-12 day intervals for up to 6 weeks to determine leptospiral growth.

(2) Agar Plates for Isolating Leptospires

A modification of the Smibert's modification of Loesche and Socransky's isolation agar plate technique was developed and utilized. Johnson's Basal Medium with 1% agar (9 vols) and rabbit serum(1 vol) was used. Small plastic (60 mm x 15 mm) dispo plates contained the agar and a .22 micron Millipore filter (sterile) was placed on the agar. A sterile plastic ring (1 in. diameter) was adhered to the filter with sterile stop cock grease. A manure specimen from the inoculated candles was placed within the ring. The plates were incubated at 30C. The filter and ring were removed from the agar plate in 4-14 days. Initially, growth of leptospires were first read at 4-6 days. Plates were held for up to 6 weeks and examined microscopically at weekly intervals for presence of leptospiral growth. (For detail protocol see Appendix A).

Identification of Isolates

Following isolation in cultural medium leptospires were subcultured and filtered to remove contaminants and preliminaryly identified by the microscopic agglutination (M.A.) tests against specific pomona hyperimmune serum produced in rabbits.

Survival and Detection of Leptospires in the Laboratory Oxidation Ditch at Winter Temperatures.

The following survival studies were conducted to determine the ability of leptospires to survive in the environmental conditions of Model A summer and winter temperatures as occurs in Northern climates. These studies were conducted with leptospires seeded in Selas candles suspended in the manure of the oxidation ditch in the laboratory, and seeded directly in the manure of the ditch, and seeded in the sludge and effluent of settling chambers.

The following experiments were conducted at winter environmental temperatures:

1. Selas candles, 6 experiments No. 1LW, 2LW, 3LW, 4LW, 5LW, 6LW (Appendix A).
2. Settling chambers, 3 experiments No. 7LW, 8LW, 9LW (Appendix A).
3. Direct seeding of Ditch, 1 experiment No. 10LW (Appendix A).

Survival and Detection of Leptospires in the Laboratory Oxidation Ditch Model at Summer Temperatures.

The following experiments were conducted at summer environmental temperatures.

1. Selas candles, 5 experiments No. 1LS, 2LS, 3LS, 4LS 5LS (Appendix A)

2. Settling chambers, 5 experiments No. 6LS, 7LS, 8LS, 9LS, 10LS (Appendix A)

3. Direct Seeding of Ditch, 2 experiments No. 11LS, 12LS (Appendix A)

Miscellaneous Studies

One study (1A) was conducted at a mean temperature of 12.6C This temperature approximated spring or fall ambient temperatures.

A study was conducted to measure survival and detection of leptospires seeded in unchlorinated well water collected from a well at the Rosemount Agricultural Field Station and in a natural stream water collected from a stream located in the vicinity of the Rosemount oxidation ditch.

Results

All attempts to isolate or detect leptospires in manure from field ditch samples (prior to adding to model ditch) were negative.

Methods of Detecting Leptospires

1. Fluorescent antibody staining (FA) to detect leptospires.

A satisfactory method for the FA procedure was developed and utilized. Leptospires were observed by FA in the manure for the length of time in which survival was measured culturally or for longer time periods.

2. Darkfield Microscopy

Leptospires were identified by darkfield microscopy of the specimen examined. These findings were consistant with the FA findings and many times of longer duration, than the survival which was measured culturally. Initially, motility of leptospires was observed with a subsequent decrease until non-motility was observed. Finally fragmentation and disintegration were noted.

Methods of Measuring Survival

1. Tube dilution procedure for measuring leptospires.

Serial dilutions of manure samples, 1/1,000, 1/10,000 and 1/100,000 in culture medium were found most satisfactory for diluting contamination and measuring survival. Some inoculated tubes, especially in lower (1/10 - 1/100) dilutions were discarded prior to the 6 week reading as a result of contamination over-growth. In later experiments only 1/1,000--1/100,000 dilutions were used, thus saving time, space and culture medium.

2. Agar plates for isolating leptospires

Isolation of leptospires were made using this technique during summer environmental temperatures, but attempts to isolate at winter temperatures were negative.

Refer to Table I, Appendix A, for results and comparison of data on the survival and detection of leptospires under winter environmental conditions.

Experimental details of daily examinations under specified environmental conditions are given for studies in Selas candles, the settling chamber and direct seeding in the ditch in the Tables (Appendix A). Note that daily readings, for example, pH, D.O. and temperature are found in the tables. Those figures are averages of 4 readings recorded each 24 hours during the time of experiments.

Sampling for detection or measuring survival was conducted once each day during the time in which the experiment was conducted.

Survival and Detection of Leptospires in a Model Oxidation Ditch at Winter Temperatures

1. Leptospiral survival studies in Selas candles suspended in the manure in ditch Model A.

Experiments 1LW, 2LW, 3LW, 4LW, 5LW, 6LW (Appendix A) are summarized in Table IA. The maximum survival time measured was 11 days (Exp. 6LW). Survival was measured until termination of the experiments. No survival was measured in Exp. 3LW. The survival time of leptospires in Candle C in Exp. 4LW, 5LW, and 6LW was considerably longer than control candles A and B. With the exception of one isolation made in (control) Exp. 1LW, the agar plate method of isolation was found to be of no value at winter temperatures. Leptospires were detected by darkfield microscopy and FA staining in nearly all candles until termination of the experiments.

When the mean pH was 9 or higher, (compare Exp. 1LW, 2LW, and 3LW to Exp. 6LW), the measured leptospiral survival time was

TABLE IA. SUMMARY OF SURVIVAL AND DETECTION OF LEPTOSPIRES IN A LABORATORY OXIDATION DITCH AT WINTER TEMPERATURES.

In Selas Candles

Exp No.	Days Conducted	Mean Temp (C)	Mean pH	Mean D.O. (ppm)	Survival and Detection in Days: Tubes	Plates	Darkfield	FA
1 LW	8	2.9	9.2	13.4	1	0	8	8
2 LW	9	1.7	9.0	13.4	1	0	9	9
3 LW	8	2.3	9.0	12.7	0	0	8	8
4 LW	9	2.1	8.4	7.3	8	0	9	9
5 LW	7	2.7	8.4	6.4	7	0	7	7
6 LW	12	2.4	8.2	5.4	11	0	11	11

In Settling Chambers

Exp No.	Days Conducted	Mean Temp (C)	Mean pH	Mean D.O. (ppm)	Tubes T*	Tubes M*	Tubes B*	Plates T	Plates M	Plates B	Darkfield T	Darkfield M	Darkfield B	FA T	FA M	FA B
7 LW	9	3.4	8.7	3.5	9	9	8	0	0	0	9	7	9	9	9	9
8 LW	7	2.9	8.3	4.6	7	7	7	0	0	0	7	7	7	7	7	7
9 LW	12	2.7	8.2	6.8	11	11	11	0	0	0	11	11	11	11	11	11

Directly in Ditch

Exp No.	Days Conducted	Mean Temp (C)	Mean pH	Mean D.O. (ppm)	Survival and Detection in Days: Tubes	Plates	Darkfield	FA
10 LW	26	3.1	6.9	7.0	(22) (18 days - post seeding)	0	25	24

*KEY
T = Top - effluent
M = Middle - effluent
B = Bottom - sludge

shortened.

During winter environmental studies, great difficulty was encountered in maintaining the D.O. manure environment below 5 ppm. Greater than 5 ppm D.O. was measured with the rotor stopped for a period of time. Shorter survival times were measured when D.O. was measured at more than 10 ppm.

2. Leptospiral survival in effluent and sludge of Model Settling chamber

Procedures used in these studies (Exp. 7LW, 8LW, and 9LW, Appendix A) were summarized in Table IA and were identical to those conducted during summer environmental studies except that winter temperatures were maintained.

The maximum survival measured was 11 days or the duration of the experiment conducted in effluent and sludge of Exp. 9LW.

3. Leptospiral survival studies in the Model A ditch

Approximately 37.5 billion leptospires were seeded directly into the ditch liquid media daily for 5 days. Results are found in Exp. 10LW, Appendix A and summarized in Table IA. After the first day of seeding, leptospires survived for 22 days (18 days after 5th, final, day of seeding) in the sludge and the liquid media of the ditch. No isolations were made using the plate procedure. Leptospires were observed for 26 days by darkfield microscopy and 24 days by FA. The pH mean was 6.9 and D.O., 7. These environmental conditions may predispose the leptospires to longer survival.

The agar plate isolation method was found ineffective at winter environmental temperatures. At this cold temperature a lag time was observed in the growth phase in using the tube method. While it was necessary to observe the cultue tubes as long as 6 weeks, growth established in 4-7 days (summer) on the agar plates. In both the darkfield and FA methods of detection, leptospires were detected until the experiment was terminated. The pH ranged from 8.2 to 8.7 and the D.O. was about 5 ppm.

Survival and Detection of Leptospires in Model Oxidation Ditch at Summer Temperatures.

Refer to Table IIA for results and comparison of data on the survival and detection of leptospires under summer environmental conditions. Table IIA summarizes the experiments. The individual tables of experiments containing the daily observations made in Selas candles, direct seeding in the ditch, and seeding in the effluent chambers are found in Appendix A.

TABLE IIA SURVIVAL AND DETECTION OF LEPTOSPIRES IN AN OXIDATION DITCH AT SUMMER TEMPERATURES.

In Selas Candles

Exp No.	Days Conducted	Mean Temp (C)	Mean pH	Mean D.O. (ppm)	Survival and Detection in Days			
					Tubes	Plates	Darkfield	FA
1 LS	13	24.3	8.4	ND	0	0	13	ND
2 LS	6	19.3	8.4	1.1	0	ND	5	3
3 LS	13	19.2	8.6	2.8	2	1	11	13
4 LS	8	19.3	7.9	4.0	1	1	5	1
5 LS	13	18.8	7.0	5.0	6	1	13	12

In Settling Chambers

Exp No.	Days Conducted	Mean Temp (C)	Mean pH	Mean D.O. (ppm)	Survival and Detection in Days											
					Tubes			Plates			Darkfield			FA		
					T	M	B	T	M	B	T	M	B	T	M	B
6 LS	8	20.0	ND	ND	ND			ND			8	8	3	8	8	8
7 LS	4	19.2	8.7	7.7	2	2	0	0	0	0	4	4	4	4	4	4
8 LS	5	18.8	9.0	8.9	5	5	4	0	0	0	5	5	3	3	3	1
9 LS	6	20.0	ND	ND	0	0	2	0	0	0	6	2	1	6	6	1
10 LS	7	18.8	8.5	9.2	0	0	0	0	0	0	7	3	2	7	7	7

Directly in Ditch

Exp No.	Days Conducted	Mean Temp (C)	Mean pH	Mean D.O. (ppm)	Survival and Detection in Days			
					Tubes	Plates	Darkfield	FA
11 LS	24	18.8	7.9	2.9	0	0	10	11
12 LS	-	20.1	6.4	7.7	1	138	4	0

KEY
ND = Not Done
T = Top - effluent
M = Middle - effluent
B = Bottom - sludge

The studies conducted in the Selas candles are reported in Table IIA and Experiments 1LS, 2LS, 3LS, 4LS, and 5LS (Appendix A).

In these experiments leptospires survived in candle C for a maximum of 6 days by the cultural method (Exp. 5LS), and no survival in Exp. 1LS and 2LS. Comparing candle C with control candles A and B, the leptospires survived longer in candle A, containing leptospires and the phosphate buffer. In Exp. 5LS the survival time measured was found identical in candles A, B, and C.

Intact leptospires were detected by darkfield microscopic examination and the fluorescent antibody staining for the duration of the study (Table II, Exps. 1LS and 5LS). Isolation procedures in some instances failed to measure viable leptospires. It is significant that intact leptospires existed in the manure environment for such long periods of time. The darkfield examination of manure samples for leptospires is a difficult procedure by which to evaluate viability. Although motility may be observed, darkfield examination is not a definitive measurement of survival. Leptospires were observed as non-motile, but when inoculated into cultural medium, growth and subsequent motility were observed. Due to the presence of many kinds of microorganisms and artifacts the validity of identifying non-motile leptospires is questionable.

Longer survival (6 days) was measured at neutral pH (Exp. 5LS) than at alkaline pH (Exp. 3LS), (2 days). The effect of D.O. is noted when Exp. 2LS and Exp. 5LS are compared, with no survival measured in Exp. 2LS with a D.O. of 1.1 ppm and 6 day survival measured in Exp. 5LS with a D.O. of 5.0 ppm.

Leptospiral experiments on leptospiral survival in effluent and sludge of the model settling chamber.

The maximum time of survival measured in the effluent was 5 days and sludge 4 days (Exp. 8LS). Unfortunately, this experiment was terminated on the 5th day. In Exp. 9LS and 10LS, survival was not detected in the effluent in a 7 day period but was detected in the sludge in the 3rd day (Exp. 9LS) in most instances (Table IIA). Darkfield and FA techniques detected intact leptospires for the duration of the experiment. The survival of leptospires in Exp. 8LS effluent and sludge existed under an extreme alkaline pH of 9.

A mean D.O. greater than 5 was measured in Exp. 7LS, 8LS, and 10 LS. The higher D.O. may be explained by the presence of H_2S gas, produced under anerobic conditions, resulting in inaccurate D.O. determinations. Although the settling chambers were not aerated, D.O. readings were recorded higher than in Model A (aerated) experiments (Table IIA, Appendix), reason, undetermined.

(Table IIA, Exp. 11LS) Surviving leptospires were not measured in the mixed liquid manure or in the sludge. Being unable to measure viable leptospires may have been due to the rapid death of leptospires in the manure environment or failure of laboratory procedure to measure survival.

Leptospires were detected in the manure by darkfield microscopy for 10 days and by FA techniques for 11 days. Based on previous survival experiments the D.O. level and pH appeared adequate for survival, but no survival was measured by the cultural technique.

Near the end of the project a repeat experiment of seeding the laboratory model ditch (summer) was conducted. (Exp. 12LS, Appendix A). In this experiment only the rabbit serum agar plate method of isolation was used. Isolation attempts were conducted daily for 23 days and thereafter intermittently. Manure (113 liters) was added to the model ditch only at the beginning of the experiment.

Leptospires were cultured and isolated on the 82nd day post seeding. Leptospires were isolated at all 6 sampling sites on 138 days postseeding by the plate culture technique. Morphological identification was made by Darkfield microscopy. These isolates were lost in sub-cultural techniques and definitive identification was not made.

Miscellaneous Experiments Conducted on Leptospiral Survival

Temperature : Spring - Fall

One experiment (No. 1A) (Table IIIA) was conducted at a mean temperature of 12.6C. This temperature is common in the fall and spring seasons. Leptospires were found to survive for 5 days by the tube culture procedure and 7 days by the plate culture technique. Leptospires were detected for the duration of the experiment by the FA technique.

Leptospiral survival studies in Stream and Well Water from Rosemount Ditch Area. (Table IVA)

Studies were conducted to determine the length of leptospiral survival in Rosemount stream and well water. Three million leptospires were placed in a Selas candle that was placed in well water. In another experiment 1.5×10^{10} leptospires were placed directly into well and stream water held in containers. Preliminary survival in water studies were conducted at summer temperatures. Survival in well and stream water was measured to be 2 days. Leptospires were detected for longer periods of time by darkfield and fluorescent antibody techniques. The tube culture and plate agar techniques were comparable in measuring

Table IIIA SURVIVAL AND DETECTION OF LEPTOSPIRES IN ANIMAL MANURE DISPOSAL

Day	Manure Added (lb)	Manure Environment				Barometer Mean (in.)	Survival and Detection Measurements								
		pH Mean	Ditch Temp. Mean (C)	D.O. Mean (ppm)	Total Solids (ppm)		Tubes Candle			Darkfield Candle			PA Candle		
							A	B	C	A	B	C	A	B	C
Experiment No. 1A Candle Studies at 11.5 - 13C.															
1	2.2	8.5	13.8	ND	ND	ND	ND	ND	ND	++	++	++	ND	ND	ND
2	2.2	8.5	12.5				P	P	P	++	++	++	+	+	+
3	2.2	8.4	13.5				P	P	P	+	++	+	+	+	+
4	2.2	8.5	13.0				P	P	P	++	++	++	+	+	+
5	2.2	8.3	12.8				P	-	P	ND	ND	ND	+	+	+
6	2.2	8.5	12.1				TP	-	TP	++	+	++	+	+	+
7	2.2	8.5	13.0				-	-	-	++	-	++	+	-	+
8		8.6	12.8				P	-	P	+	+	+	+	+	+
9		8.8	13.2				-	-	-	++	-	++	+	+	+
10		8.9	12.3				-	-	-	+	+	+	+	+	+
11	2.2	8.8	13.0				-	-	-	+	+	+	+	+	+
12	2.2	8.7	12.0				-	-	-	+	-	+	+	+	+
13	2.2	8.8	11.9				-	-	-	+	+	+	+	+	+
14	2.2	8.8	13.4				-	-	-	+	+	+	+	+	+
15	2.2	8.8	12.1				-	-	-	+	+	+	+	+	+
16	2.2	8.7	12.0				-	-	-	+	+	+	+	+	+
17	2.2	8.7	11.9				-	-	-	+	+	+	+	+	+
18	2.2	8.9	11.8				-	-	-	+	-	+	+	+	+
19	2.2	8.9	12.9				-	-	-	+	-	-	+	+	+
Mean		8.7	12.6												

T = tube
P = plate

++ = motility

TABLE IVA SUMMARY OF SURVIVAL AND DETECTION OF LEPTOSPIRES IN WELL WATER AND STREAM WATER.

Exp No.	Days Conducted	Mean Temp (C)	Mean pH	Mean D.O. (ppm)	Survival and Detection in Days			
					Tubes	Plates	Darkfield	FA
7A Selas Candles in well water	5	19.9	8.6	2.7	2	2	5	5
8A Selas Candle in stream water	9	10.4	7.9	4.0	2	2	5	1
10A	9	ditch 18.6 Bea Kup 19.0	ditch 7.9 Bea Kup 8.3	ditch 5.7 Bea Kup 5.8	A & B 2	A & B 2	A & B 8	A & B 4

KEY
A = Well
B = Stream

survival in water.

Additional Observations

1. The laboratory Model A which contained 113 liters of manure was housed in the departmental laboratory and found to be nearly odorless when aerated to maintain a D.O. of 5 ppm.

2. During the year, predominantely during summer environmental temperature studies, foaming was found to be a major problem which occurred especially when the ditch was being stabilized. During this time foaming also occurred in the field ditch. After the rotor was changed from a brush to a paddle type, foaming decreased and was no longer a problem.

3. At summer environmental ditch temperatures, active degradation of manure occurred in the laboratory model.

4. In February, 1970, four feeder cattle (approximately 1100 pounds average weight) were found dead over the Rosemount oxidation ditch. Following necropsy examination, a tentative diagnosis of idiopathic toxicosis was made since prior to the death the rotor had failed to function for hours and death followed start-up. Consideration of possible emission of toxic gases from the manure located in the oxidation ditch was made.

Discussion

The laboratory model of the field oxidation ditch developed was found to be an adequate environment for leptospiral survival and detection studies of leptospires under simulated winter and summer temperature conditions. It should be emphasized that these conditions simulate, but did not duplicate, the environmental conditions (pH, D.O., temperature, and total solids) that existed in the operational field ditch unit. When the D.O. was maintained at 5 ppm or greater in front of the rotor, the manure of the model ditch was essentially odorless in the laboratory. Under winter conditions difficulty was encountered in maintaining the D.O. at 5 ppm in front of the rotor. The rotor speed was reduced to minimize the aeration. Daily additions of small amounts of manure caused a lowered pH of ditch manure.

For measurement of survival the results indicated that the tube dilution isolation technique was found to be the most adequate under winter conditions. In addition to problems of contamination, the modified agar plate technique was found unsatisfactory for isolation of leptospires, for at cold temperatures (1.7 - 6.4C), there may have been a lag in the leptospires ability to penetrate the filter to establish growth in the agar medium. Using this method, no isolations were observed during winter studies.*

In the leptospiral survival studies under simulated summer environmental conditions, the beef cattle manure in the laboratory model system of the Rosemount field oxidation ditch was adequately utilized. In the laboratory methods of isolation, the tube dilution procedure was utilized for culturing and growth of leptospires from the manure. However, overgrowth of contaminants was a problem and may have resulted in failure to measure maximal survival time of the leptospires. The modified agar plate method was utilized in filtering out contaminants and isolating leptospires. Findings in Experiment 12LS (Table IIA) indicate that the changes in procedure resulted in a longer term and more accurate determination of survival time.

For detection, the fluorescent antibody procedure developed was adequate for identifying the presence of leptospires in the manure for a period equal to or greater than the cultural isolation. Controls were essential due to a large amount of extraneous materials and possibility of reading false positives.

Darkfield examination was found adequate for detection and measuring motility of leptospires. This procedure requires a skilled observer to differentiate leptospires from artifacts and other numerous microorganisms. Darkfield is generally unreliable when the leptospires become non-motile. This examination requires a trained, skilled observer. Neither of the above methods definitively measure survival.

The survival time (Table IA) of 18 days in the ditch was the maximum time measured (Exp. 10LW). A mean pH of 6.9 was observed. In comparison, survival studies in Selas candles (1LW, 2LW) with the pH of 9.0 - 9.2, the survival time was measured to be one day. The leptospires survived for a longer time in the aerated ditch than in the settling chamber (sludge and effluent).

In the settling chamber the survival time was measured for a maximum of 11 days in both effluent and sludge. Unfortunately, these tests were not continued for a longer time period.

In Table IIA measurements indicated that leptospires survived for at least 6 days in the manure in Selas candles, for 138 days (82 day definitively) in the manure of the oxidation ditch model , and for 5 days in effluent and sludge of a settling chamber in experiments conducted at summer environmental temperature. Leptospires survived only 3 days in well and stream water.

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*In later studies of leptospiral survival in beef cattle manure at winter temperatures, the plate agar technique, following additional modifications was very effectively used for isolation.

Although *Leptospira pomona* had been isolated for about 18 days in winter studies utilizing the tube dilution procedure, developments in cultural isolation techniques utilizing the agar plate technique allowed for isolation of *L. pomona* for approximately 138 days under summer conditions. These isolations were relatively routine regarding ease of isolation and the number of organisms present indicates that the leptospires not only survived but may have also muliplied in the model oxidation ditch manure environment. Additional research is needed to definitively quantitate and document leptospiral multiplication.

These findings indicate a greater survival time of leptospires in aerated liquid manure of the model oxidation ditch than in effluents or sludge, indicating that aeration provides a more suitable environment for survival. Further studies are needed.

Little research has been conducted on the survival of pathogenic leptospires in animal manures. One report indicated that *L. icterohaemorrhagiae* could not survive in human feces for more than 24 hours and that polluted water, sewage, and soil will not keep *icterohaemorrhagiae* alive for more than 3 days (41).

Reports indicate that pH less than 5.0 or greater than 8.5 is detrimental to leptospires. The optimum appears to be 7.2 - 7.4 (42).

Other researchers have indicated that leptospires survive in the environment for varying lengths of time. Chang, Buckingham and Taylor (32) found that leptospires survived in river water 8 - 9 days at 5 - 6C, 5 -6 days at 25 - 27C, and 3 - 4 days at 31 - 32C. Indications were that lower temperatures were more conducive to survival. It was also indicated that presence of other microorganisms probably was detrimental to leptospires. In domestic sewage,survival of leptospires was only 12 - 14 hours but rose to 2 - 3 days when aerated. Survival was 7 - 8 days when sewage was diluted with tap water to 1% of its strength. Survival was measured for 6 - 7 days in 10% sewage in tap water at 5 - 6C (pH 7.1 - 7.2).

Ryu and Liu (33), in laboratory viability studies with leptospires, attempted to define a survival pattern in rice-planted paddy water, found a decreasing survival time at high temperatures (40 - 43C, viable 3 - 6 hours)as compared to low temperatures (0 - 30C, viable 7 - 14 days).

In environmental studies Okazaki and Ringen (43) reported that temperatures below 7 to 10C or above 34 to 36C, and pH below 6.0 or above 8.4 were detrimental to survival of serotype pomona. Seroytpe pomona survived for less than seven days under simulated natural conditions, and longer in stagnant pools, than in moving water. Addition of small amounts of fresh water to stagnant pools increased the organisms' survival time. In soil studies leptospires survived for 30 minutes in air-dried soil, 3 - 5 days in damp soil and 183 days in soil super-saturated with water.

In studies of four leptospiral strains in Malaya, Smith and Turner (44) reported that leptospires survived longer in alkaline than in acid buffered distilled water, and significant differences between the four serotypes were found in resistance to pH. Survival at pH under 7.0 ranged from 10-117 days and at pH over 7.0 from 21-152 days.

The aerated beef cattle manure, effluents, and sludge appeared suitable for survival of leptospires. Infected cattle may shed leptospires for several months in the urine. Up to 100, 000,000 leptospires per ml. of urine have been reported (31). The finding of viable leptospires in the aerated ditch for more than 138 days in the summer and for 18 days in the winter is significant. A critical need exists to define whether or not the surviving leptospires have maintained virulence to infect animal hosts. The oxidation ditch containing beef cattle manure constitutes an adequate environment for the survival of pathogenic leptospires of significantly long duration. Such contaminated manure hauled to the land, or effluent may be discharged into natural waters, thus constituting a potential health hazard.

SURVIVAL AND DETECTION OF SALMONELLA IN BEEF CATTLE MANURE.

Introduction

Epidemiologic surveillance indicates that we have not controlled animal-associated salmonella. In the United States there are an estimated 2 million human cases annually. (45)

There is increasing concern for the salmonellosis problem in both man and animals. In 1970 there were 24,216 human cases of salmonellosis (excluding typhoid fever) reported in the United States with 335 in Minnesota. Salmonella organisms have been found in surface waters contaminated by animal manure. In 1966, a large waterborne outbreak at Riverside, California resulted from contamination of the water supply by *Salmonella typhimurium*.

This serotype is widely found in domestic animals. There is speculation that the water may have been contaminated by seepage from distant cattle feedlots (14). From June, 1964-October, 1965, an investigation of water pollution along the Upper Mississippi River and its major tributaries was conducted in Minnesota and Wisconsin. Pathogenic bacteria and viruses were isolated from stream and waste samples. Fourteen species of *Salmonella* were found in sewage effluent at a sewage treatment plant. Several species of *Salmonella* were found in the river at locations six and ten miles downstream (46). Salmonellae have been isolated in influents and a considerably lesser amount in unchlorinated effluents of the Municipal Sewerage Plant at Glenwood, Minnesota, which utilizes the oxidation channel method. Chlorinated effluents were not examined for *Salmonella* (47).

The single-host species of typhoid and paratyphoid organisms are being encountered less frequently as the etiologic agent of human suffering. However, the ubiquitous, multi-host salmonellae are being isolated from warm and cold-blooded animals, and as a contaminant from food, feeds, and numerous other materials. The infections they cause have been reported more often, especially in persons very young, very old, or debilitated. Wherever ecologic research and investigation probe into our environment, salmonellae are found.

Although 1,300 distinct serotypes of unadapted salmonellae exist, 96% of the cultural isolates from animals and man belong to only 55 serotypes. *Salmonella typhimurium* is the salmonella most common to infect man and domestic animals.

Although 95% of Americans live and associate essentially in urban areas, man and animals do share a common environment

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for work and recreation. There is a tremendous interaction between host, pathogenic agent, and environment. It is difficult to comprehend this interplay because many factors in the disease transmission remain unknown.

How will salmonellosis be controlled? Generally speaking, immunization or prevention of exposure are the two methods used to protect against infection. And yet, vaccination against salmonellosis appears inefficient and ineffective (48, 49, 50, 51) and drug resistance studies indicate that salmonellae cannot be eliminated with chemotherapeutic agents (45 , 48). For the most part, even the minimum infective dose is not known as there are so many types and strains of salmonella. Therefore, the main thrust in efforts to control salmonellosis must be to decrease the exposure potential within our society. But, as populations grow, animals, as well as people, both of which carry salmonella, are often found living in crowded conditions.

Economic pressure has forced producers to cloister livestock in large numbers into more and more congested housing which often does not satisfy basic sanitation requirements. Cattle may harbor and excrete salmonellae in feces, and subsequently pulverize this waste which becomes airborne as dust laden with the microorganisms and spread to other areas. Or, the salmonella-laden manure accumulates until such times when it is washed by land run-off into streams, rivers, lakes, and on through the water ways to pollute harbors and estuaries. Wild animals, fish, mollusks, and other lower animal forms living in and near this environment may become infected (52, 53). Furthermore, this is the common environment in which we grow food and seek recreation.

Cattle afflicted with salmonellosis may shed 10 million microorganisms per gram of feces (54).

The principle of the oxidation ditch is to maintain manure as suspended solids of biological value in water of sufficient oxygen content to maintain an aerobic condition allowing for microbial degradation. This is accomplished within an end-to-end channel through which the manure is propelled by a rotor which serves to aerate the material. (Figure 1). The aerobic process is nearly odorless. These two factors thus facilitate handling and reuse.

After a period of time, the waste from an oxidation ditch field unit can be disposed of in a number of ways. It may be mechanically pumped from the ditch into a tank truck and then spread onto land. Or, the effluent may be discharged into an irrigation channel or stream. Studies are being conducted to ascertain the feasibility of recycling cattle and poultry manure wastes as feed for domestic animals (55, 56) and at a profit, too.

British investigators recently reported survival of *Salmonella typhimurium*, *S. dublin*, *Staphylococcus aureus*, *Escherichia coli*, and *Brucella abortus* in cattle manure slurries for 12 weeks (57).

Methods

In order to evaluate the potential health effects of salmonella in cattle manure, research was conducted in a laboratory model oxidation ditch and effluent holding chambers simulating field environmental conditions at summer and winter temperatures. The objectives of the research herein reported were: to measure salmonella survival time, to develop and improve bacteriologic methods of measurement of detection and survival of pathogens in beef cattle manure.

All field samples of manure were examined for *Salmonellae* prior to adding to the model. During experiments, DO, pH, and temperature data were monitored at 6-hour intervals.

The 2C manure temperatures were adequately maintained by the cooling system as originally conceived. This cooling system consisted of condensor, fan, cooling coil, and channel trough, antifreeze, holding vat, and thermostat. However, on or about April 27, 1970, it was discovered that the refrigerant in the open channel coolant trough was leaking into the model ditch manure, thus killing the microorganisms. The trough was then replaced with refrigerator coils/ tubes which made the cooling/coolant system a closed system. This redesign prevented further known leakage and did not affect the salmonella experiments.

The 20C (summer) manure temperatures were difficult to maintain. When the ambient air temperature was near 20C the ditch manure would acclimate with it. The warm-to-hot Minnesota summer temperatures which frequently range as high as 32C and above outdoors, cause equally as high or higher indoor temperatures in the lab, thus precluding maintenance of the 20C ambient temperature without the aid of a room air conditioner for the lab. It had been hoped that the cooling system on the model A unit would be able to maintain 20C, but its control range would allow only 17-18 degrees as a controlable maximum temperature. The problem encountered was that the air conditioning unit, though of adequate capacity for the room-size, did not function well enough. Occasionally it would overload the lab circuit and shut off. Periods of high relative humidity tended to cause the air conditioning unit to ice up, especially if the daytime temperatures were extremely hot and the nightime temperatures 10C cooler. As a result, the summer oxidation ditch A manure temperatures were more fluctuating than the winter temperatures, even though on average both winter and summer manure temperatures were close to 2C and 20C as specified. Styrofoam insulation placed about the outside periphery of the 2C ditch did aid to maintain proper temperatures during June, July, and August. Ice would build-up on the refrigerating coils of the model oxidation ditch.

Through prolonged continuous use, the bushing of the paddle wheel assembly, which was constructed of ordinary steel would wear to a point whereby the functional capacity of the paddle was not adequate. The bushings were replaced with lubricant-impregnated brass which provided a longer wearing potential than the original bushings.

The Effluent Chambers/Flask (Experiment 27B)

Studies of Salmonella typhimurium survival in effluent was considered necessary in light of two facts. First, the Pasveer oxidation channel can be operated on a batch load-unload basis or as a continuous skim-off system whereby effluent is continuously being discharged from the ditch. Second, consideration of the material itself, which is a suspension of solids in liquid, indicated a biphasic system whereby both phases, effluent and sludge, required testing.

Effluent chambers were designed to contain 1 liter of aerated ditch waste were utilized in experiments 27B, 29B and 29C, and experiment 30B. The waste was allowed to settle and the effluent (top) and sludge (bottom) were sampled to detect Salmonella typhimurium. Monitoring of the pH, D.O., and temperature was also achieved. In experiment 29B and 29C the chambers were placed in the channel of the oxidation ditch to acclimatize with it. The chambers of experiments 27B and 30B were separate from the ditch. The sampling procedure was the same as for sampling the model oxidation ditch A. Experiment 27B was in a flask.

Contamination Studies Involving the Effluent Chambers

Contamination Testing.

During experiment #29 wherein the effluent chambers of #29B and #29C were in the stream of the model A oxidation channel, it became apparent to us that contamination of ditch manure or effluent chamber material may have occured. Two probable sources of cross-contamination between ditch and chambers were: aerosol transmission, and/or the contaminated instrument probes for the D.O., temperature, and pH determinations even though they were rinsed during the interval between samplings. Procedures were established to determine the probability of such cross-contamination. Washings from the probes were cultured for salmonella, and

TABLE 1. CALCULATED NUMBER OF SALMONELLA TYPHIMURIUM SEEDED INTO MANURE.

Experiment No.	Day	No. Salmonella
26 A	0	33×10^6
	1	33×10^6
	2	33×10^6
	3	33×10^6
	4	33×10^6
27 A	0	33×10^6
	1	33×10^6
	2	33×10^6
	3	33×10^6
	4	33×10^6
27 B	0	3.0×10^7
28 A	0	33×10^6
	1	33×10^6
	2	33×10^6
	3	33×10^6
	4	33×10^6
29 A	0	33×10^6
	1	33×10^6
	2	33×10^6
	3	33×10^6
	4	33×10^6
29 B	0	3.0×10^7
	1	3.0×10^7
29 C	0	1/113 of 2 day seeded ditch manure
30 A	0	33×10^6
	1	33×10^6
	2	33×10^6
	3	33×10^6
	4	33×10^6
30 B	0	1/113 of 5 day seeded ditch manure

S-S (Salmonella-Shigella) and BGS (Brilliant Green Sulfadiazine) plates were exposed for 24 hours at three locations in the model ditch, that is, on each of the two effluent chambers, and atop the rotor housing.

All results from the washings and the exposed plates were negative. It was decided, however, to isolate the effluent chambers from the model A oxidation ditch in further studies by separating them. Thus, during experiment #30, which was conducted at 2C, the effluent chambers were kept stable at 2C in refrigerators, and not monitored for pH, D.O. and temperature once seeding of either ditch or chambers had occured.

During salmonella experiments #26-30 additional manure was introduced into the oxidation ditch on two occassions. The physical data recorded from the model oxidation ditch in experiments #26, #27, and #28 indicated little or no change in the parameters after the addition of the manure to the original slurry. Therefore, it was decided to not add extraneous manure after the original charging load in subsequent experiments to eliminate as much as possible any undetermined error. Experiment #29 was conducted for at least 15 days, and experiment #30, for 28 days longer than previous experiments.

The three parameters, D.O., pH, temperature, were consistantly comparable one to the other. Thus, it was felt that the addition of manure increments after the initial charge did not contribute considerably to the physical make-up of the waste, and neither added nor subtracted from the quality of the micro-biological climate of the material. Although salmonella organisms were detected in the manure for periods longer than those in the manure experiment #29 and #30, the longer survival times were considered to be the 2C degree winter ditch temperature, as well as the improved cultural detection. The samples were not only processed in a manner different from original cultures, but also more samples per sampling site were analyzed routinely, that is, more data were recorded.

Maintenance of Conditions

Model Oxidation Ditch

The oxidation ditch model containing salmonella-free well water and manure from Rosemount field unit was stabilized with regard to pH, temperature, dissolved oxygen, total solids, and mechanical operation initially before being seeded with *Salmonella typhimurium* stock culture. On two occasions, *Salmonella* was detected in the manure and these lots were discarded. Throughout each experiment, efforts were made to maintain the pH between 6.5 and 8.0 the total solids between 5,000 and 10,000 mgs/liter, the dissolved oxygen at 5 ppm., and the temperature near 20C for summer conditions, and 2C for winter conditions. These conditions were achieved by regulation of temperature alone for the most part.

Effluent Holding Chambers/Flasks

The monitoring of pH, D.O., and temperature in the chambers incorporated the same probes used in monitoring the oxidation ditch.

Temperature was the only parameter maintained in the effluent holding chambers/flasks. Accurate monitoring and recording of pH and dissolved oxygen was accomplished in experiments #29B and #29C, and nearly identical conditions are assumed for experiment #30B.

Seeding of Salmonella

Stock cultures of biochemically and serologically pure *Salmonella typhimurium* grown on tryptic soy agar were inoculated into biphasic veal infusion broth agar (1-2.5%) for cultivation. These cultures were again screened for *S. typhimurium* and utilized for direct seeding into either the oxidation ditch or the effluent holding chambers of experiments #27B (Flask) and #29B.

Calculations on the basis of reference (54) indicated that 5-6 billion organisms daily were required for seeding the model ditch to simulate the Rosemount operational oxidation ditch at maximum *S. typhimurium* shedding from the 36 beef cattle there. However, such a high estimate of salmonella load was based on the assumption that the herd consisted of "scouring" calves with severe,acute diarrhea. In practice fewer *Salmonella typhimurium* would be expected. The model oxidation ditch A was inoculated daily with approximately 33 million *Salmonella typhimurium* organisms (Table 1). The number of *S. typhimurium* in veal infusion broth-agar was estimated by counting on a Petroff-Hauser grid the number of organisms per one milliliter of 30 nephlos Salmonella in VIB on a nephlometer. The inoculum was then diluted with 100 mls of sterile veal infusion broth for mixing with the oxidation ditch manure. During each experiment the laboratory oxidation ditch was seeded on the five initial consecutive days with the calculated number of *Salmonella typhimurium*.

The effluent holding chambers were inoculated either by

PETROFF-HAUSER COUNTING CHAMBER
FOR DETERMINATION OF BACTERIA *
A MODIFICATION.

The *Salmonella typhimurium* were motile and viable when *used with this method*, that is, not stained.

Salmonella

1. The number of bacteria in 20 squares of the chamber were counted, and a mean calculated.

2. The squares on the grid of the counting chamber are 1/20mm by 1/20mm and the chamber is 1/50mm deep.

3. Therefore, by multiplying the mean of 20 squares by 20 million (20x20x50x1000) the number of bacteria were calculated.

Salmonella: Veal Infusion Broth culture suspensions of 30 nephelos were utilized.

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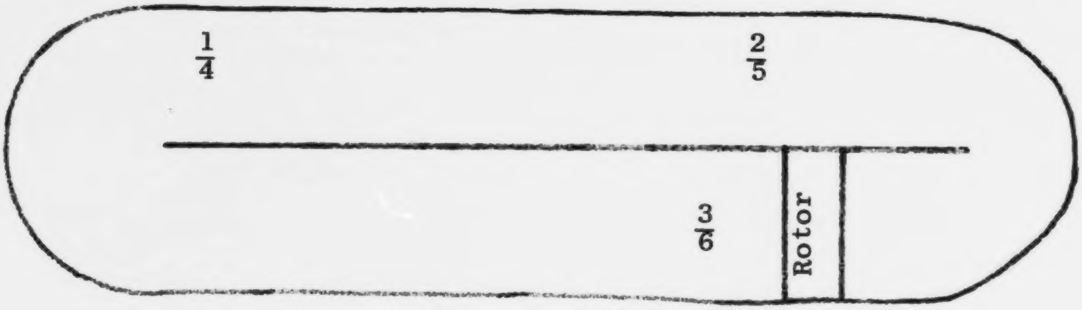
*Simmons and Gientzkow: Laboratory Methods of U.S. Army, 1944, p. 404.

directly seeding 3.0×10^7 *S. typhimurium* into the chamber containing 1,000 ml of dilute manure, (experiments 27B and 29B) or indirectly by placing 1,000 ml of seeded oxidation ditch manure into the effluent chambers (experiments 29C and 30B).

Sampling

Six sampling sites were selected at positions about the periphery of the model oxidation ditch A as indicated in Figure 7 , below. Sites 1,2,3 were



areas 1 inch below the surface of the manure, whereas, sites 4,5,6 were located at the same positions as 1,2,3 but between the floor of the model to 1 inch above the floor. The Moore (58) technique of sampling was standard protocol wherein two sterile cotton swabs were taped into position at each site and remained in place until replaced in 24-72 hours with fresh sterile cotton swabs. Personel handling contaminated material, swabs, and the like wore plastic gloves.

Modifications of the Moore procedure were tried also. In experiment #26A sterile cotton-tipped swabs were dipped momentarily into the ditch manure, then placed in enrichment media. In experiment #28 1 ml samples were taken for inoculation into enrichment media. Sampling was done daily for 1-2 weeks, then every three days for the remainder of each experiment. All samples were placed in enrichment broth prior to selective media for microbial isolation.

Methods of Measuring Salmonella Survival

Cultural Procedure

The general flow of the cultural schema was: sample to selective enrichment for 24 hours at 37.5C followed in turn by selective plating for isolation and differentiation on TSI and biochemistry. The biochemical results were verified by serological testing (Figure 6).

Original cultural methods included enrichment in tetrathionate-brilliant green medium and brilliant green (BG)-sulfadizaine medium with an incubation period of 6 to 48 hours followed by plating on Eosin-Methylene Blue (EMB), MacConkey, and Salmonella-Shigella (SS) agars. Subsequent work also utilized GN (Hajna) broth, Brilliant Green-Bile broth and Selenite-BG-sulfadiazine agar plates for isolation (see Table 2).

Differentiation was accomplished biochemically and serologically. The biochemistry utilized triple sugar iron (TSI), urea, decarboxylase base, lysine decarboxylase broth, salicin, dulcitol, and KCN broth base. H-broth and motility test media were utilized when necessary to enhance proper functioning of the serological procedure. Salmonella A and poly-B somatic antisera, and flagellar H_i and H_1 complex antisera were utilized for serotyping the isolates obtained through enrichment and biochemistry. All were commercial products.*

Serologic Procedures

TSI cultures were utilized for the serological confirmation of isolates. Difco commercial preparations were utilized in this procedure. Somatic poly A and B antisera, and later, only the B antisera, were used in the slide agglutination test to identify the isolation as to Ohnehauch heat stable antigen, Group B. Most isolates obtained through TSI and the biochemical reactions were positive at this stage with only a few rough forms being encountered. Flagella H antigen analysis was accomplished by the tube test of Edwards and Bruner (59) incorporating H_1 complex (phase 2) and H_i (phase 1) flagellar antisera.

After completion of each experiment, 5 TSI cultures of salmonella isolates which had been serologically determined in our lab as being *S. typhimurium* were randomly selected and carried to Mrs. L. York in the Department of Veterinary

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*Difco, Detroit, Michigan.

TABLE 2. SALMONELLA EXPERIMENTS: BROTH AND PLATE MEDIA INCORPORATED

BROTHS:	Tetrathionate Brilliant Green	Brilliant Green Sulfadiazine	Selenite Brilliant Green Sulfadiazine	Brilliant Green-Bile	Gram Negative (Hajna)
Experiment Number					
26	X	X			
27			X	X	X
28			X	X	
29			X	X	
30			X	X	X

PLATES:	MacConkeys	EMB	Brilliant Green Sulfadiazine	Salmonella Shigella
Experiment Number				
26	X	X	X	X
27			X	X
28			X	X
29			X	X
30			X	X

Microbiology typing lab for analysis. Mrs. York tested these isolates by the Spicer-Edwards procedure. All cultures examined were identified as *S. typhimurium* by Mrs. York. This use of allied laboratory facilities allowed not only for a check on our results but also upon the validity of our procedure.

Fluorescent Antibody Studies

The concept of being able to distinguish one genera of microorganisms from among the many others in waste, or for that matter in any environment has led the interested scientist to attempt many different approaches to attain prompt recognition of the microorganism. Among the methods available, the fluorescent antibody technique (F.A.T.) seemed to be quite promising.

The theory of fluorescent antibody action indicated a selective, sensitive method by which *Salmonella typhimurium* could be "spotted" in the midst of the myriad other microorganisms present in manure waste. It was for this reason that the FAT was considered for use in our project.

However, despite attempting various modifications of the methods of Groeffert and Hicks (60) and the National Animal Disease Lab. (61) we feel that F.A.T. as applied in our lab was not as satisfactory as the cultural methods utilized in this study. Recently a procedure (Harrington, 61) has come to our attention. This procedure , if employed, could not only improve the feasibility of F.A. for our work, but also make the cultural aspects more rapid as well. It was not applied as we have not dealt with salmonella since learning of the technique.

Difficulties with fluorescent antibody technique:

A. Non-fluorescence or repression of fluorescence.

B. Lack of readily available conjugate which we could use with confidence.

C. No one good procedure whose modifications worked well.

The direct FA technique incorporating commercially** available rabbit antiserum to Salmonella O (antigens 4,5,12) and Salmonella H_i and H_1 complex were utilized. Positive and

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**Sylvana, Millburn, New Jersey.

negative controls were tested. Conjugates were undiluted and diluted 1:4 and 1:9. These were prepared in the same manner as were the leptospiral conjugates.

Chlorination Studies

It was found from the survival studies involving both Salmonella typhimurium and Leptospira pomona that these microorganisms do indeed live on in the extended aeration processed livestock waste (manure) for a period of time. Although the salmonella experience a decimal reduction rate, which was not determined, and do become undetectable in time, the leptospires were continually detected for as long as 4 months once isolation procedures were derived, indicating multiplication rather than mere survival. Further quantitative studies are needed to confirm this belief. The implication, of course, is that, regardless of whether death occurs after a period of time or multiplication succeeds, any leakage of non-disinfected manure from the treatment/holding facilities where pathogens are present is a potential health hazard, either immediate or remote, in terms of disease transmission from a reservoir (port of exit).

Therefore, chlorination of pathogen contaminated wastes was attempted on a laboratory scale using wastes from the model A oxidation ditch. Titration of residual chlorine was used to indicate the amount of chlorine involved in the treatment of the waste, and cultural isolation was the criteria used to gauge the effectiveness of chlorination as a disinfecting method for manure wastes.

In actual fact, the slurry from the Pasveer oxidation ditch is a suspension of solids in a water vehicle and the solids portion is allowed to settle out, thus separating "sludge" waste from "effluent" waste. The two are generally handled as a single medium for spreading onto land, but more and more the two are being allowed to separate in holding ponds or the like, with the effluent portion being discharged into surface waters and only the sludge being hauled to the land as fertilizer. This surely facilitates handling because of the decreased bulk and time consumption. Pathogens exist in both portions of the waste and both must be considered potential hazards. However, often it is only the effluent which is chlorinated.

In chlorination studies, only effluent was chlorinated after it had separated from the suspended solids. Even so, the titration of residual chlorine could only be accomplished amperometrically as other methods depended upon color change which could not be visualized/detected in the turbid, green effluent. The residual chlorine determinations were performed with some difficulty. A Wallace and Tiernan Amperometric Titrator model #WIA790-1-2 was used in such titrations. The Spinning electrode agitates the sample while measuring current flow. The effluent being rich in organic matter would foam and spill out over the sample container making the quantitation of residual chlorine inaccurate.

Furthermore, after a period of time the electrode portion of the titrator cell would deteriorate to the point of leaking electrolyte solution and allowing the ingression of effluent into the cell chamber.

The procedure developed is listed on the following page.

More work is to be conducted on the chlorination of pathogen contaminated manure. Thus far we can report that a contact time of approximately 60 minutes will be necessary to destroy pathogens in the oxidation ditch effluent. Although chlorination of effluents may be accomplished, it doesn't appear that this method is practical due to the high organic load and probable NH_3 content.

Results

Salmonella typhimurium survived for 17 days post-seeding in the model oxidation ditch at summer temperatures (20C) and for 47 days under winter conditions (2C). Further, studies in model settling chambers holding 1,000 ml of model oxidation ditch effluent indicate that S. typhimurium can be detected for even greater periods of time than at similar temperatures in the oxidation ditch. The longest survival time was 87 days, experiment 5b, effluent holding chamber sludge (Table 3).

The data indicates that survival is of greatest duration in the sludge portion of the settling chambers. Information derived from the investigation of a stream gave similar results in that salmonella recovery rates for stream bottom sediments were greater than those from surface water. (62)

All isolates were biochemically and serologically identified as Salmonella typhimurium.

The ambient environmental conditions monitored were maintained within the preselected norms as determined from the field ditch (Table 4).

Based upon results obtained, the three sampling methods:

Chlorination Procedure*

1. Beef cattle manure having been processed in an oxidation ditch (extended aeration process) is allowed to separate by stasis (gravity) into sludge and effluent portions.

2. Calculations indicated that to study chlorination of the model A oxidation ditch effluent, 8.8×10^6 *Salmonella typhimurium* are required to proportionately simulate the concentration of pathogens (calculations of total pathogen content of the model A oxidation ditch, see appendix, p. 32). Salmonella is added to 195 ml of effluent which is contained in the vessel of an amperometric titrator.**

3. $Ca\ (OCl_2)_2$ at 47 mg per liter is added to this vessel.

4. Samples are incubated at room temperature for varying lengths of time (15, 30, 45, and 60 minutes).

5. The agitator is started.

6. 5 ml of phenylarsene oxide is added to the chlorinated effluent.

7. 4 ml of pH 4.0 buffer solution is then also added to the material.

8. 1 ml of potassium iodide solution is added.

9. A 0.0282N iodine solution is added from a burette to titrate the residual chlorine. The end-point is then detected on the micro-Ammeter of the titrator which is registered as a deflection of the needle of the meter.

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* from: Wallace and Tiernan, Book #WIA 790-1-2, "Determination of residual chlorine in waste-water."

** Wallace and Tiernan, model series A-790012.

TABLE 3

SURVIVAL AND DETECTION OF SALMONELLA TYPHIMURIUM IN A MODEL OXIDATION DITCH OR MODEL EFFLUENT HOLDING CHAMBERS

Experiment No.	Bacteriological Isolation	F.A.
Ditch;	Days	Days
26a	14	--
27a	14	10
28a	17	4
29a	40	--
30a	47	48
Chamber;		
27b	14	10
29b	15-effluent	10
	18-sludge	--
29c	21-effluent	--
	52-sludge	--
30b	66-effluent	--
	87-sludge	

TABLE 4

ENVIRONMENTAL CONDITIONS IN A MODEL OXIDATION DITCH AND MODEL EFFLUENT HOLDING CHAMBERS

Exp. No.	Days Conducted	Total Solids(mg/L) $\overline{x}$; range	pH $\overline{x}$; range	Temp (C) $\overline{x}$; range	D.O. (ppm) $\overline{x}$; range
Ditch:					
26a	38	6799; 6154-7256	6.7;6.1-8.5	19.4;16.0-21.0	3.8;0.4-7.4
27a	28	5747; 5317-6927	7.4;6.8-8.2	20.2;17.0-24.0	1.8;0.0-3.6
28a	38	7575; 4183-9058	7.6;6.4-8.2	20.6; 4.0-22.5	3.8;0.0-10.6
29a	53	5509; 3069-7258	8.5;8.1-8.7	1.3; 0.0-3.0	12.7;9.2-22.0
30a	66	6363; 6100-7258	7.8;6.9-8.4	2.1; 1.0-4.0	14.0;5.0-20.0
Chamber:					
27b	18	(1)	7.4; NA	20.2; NA	ND
29b	46	(1)	T 8.0;7.6-8.3 B 7.4;7.6-8.3	2.9;2.0-5.5 2.6;1.5-6.5	4.3; 0.1-9.2 3.4; 0.1-8.7
29c	54	(1)	T 8.0;7.6-8.3 B 7.7;7.3-8.4	2.9;2.0-7.5 2.6;0.0-7.0	3.8; 0.7-11.0 0.7; 0.1-4.5
30b	103	(1)	T 8.3* 7.8; B 8.2*	2.0* 2.0; 2.5*	3.8* 13.0; 1.2*

T = top ; B = bottom
NA ; not available
ND ; not done
(1); waste material separates into effluent and sludge portions which were not monitored for T.S.
* = not monitored continuously

temporary swab, prolonged swab, and increment removal, were comparable in isolation efficiency.

The greatest success for measuring survival thus far has been achieved utilizing BG-Bile and Selenite-BG-sulfadiazine as the enrichment phase, and SS and Selenite-BG-sulfadizaine the plating phase for isolation. (Table 5)

Original fluorescent antibody efforts were not as successful as anticipated. Attempts to retrieve positive flourescing S. typhimurium from the enrichment phase media were found more successful than sampling directly from the oxidation ditch manure.

During the progression of experiment 2, wherein the holding chambers were maintained in the stream of the oxidation channel, it was considered that cross-contamination from chamber-to-ditch or, conversely, from ditch-to-chamber may occur either by aerosol transmission, or from the set of monitor electrodes as only one set of instrumentation was available for monitoring both experimental subunits. Therefore, it was elected to not measure D.O., T.S., pH and temperature in experiment 28b (settling chamber) and to house the chamber within a refrigerator with the temperature held constant at 2C. The behavior of the parameters could be implied from the extensive data obtained in experiments 29b and 29c, and experiment 30b would be a control for these previous two studies. Experiment 30a (ditch) terminated prior to 30b (chamber) and one reading was made of the settling chamber manure. From the data it can be determined that chamber 30B had behaved as the chamber of experiments 29b and 29c.

Salmonella typhimurium was not detected after disinfection of the contaminated effluent with chlorine at a residual of 1.0 to 5.0 ppm. and a contact time of at least 60 minutes.

Discussion

In an attempt to simulate field conditions, salmonella research was conducted in a laboratory utilizing a model of an oxidation channel. The procedures are laboratory protocol and attempts were made to simulate, but never exactly duplicate, field conditions. This fact was exemplified in that manure was added to the model at intermittant intervals. During field conditions continous urination and defecation of beef cattle occured in the housing unit through slatted floors located immediately over the ditch. In field practice the extended aeration process of treatment is altered by the operator to meet individual needs. Most producers appreciate the nearly odorless operation and discard the

TABLE 5. DETECTION OF *SALMONELLA TYPHIMURIUM*

PLATES

	No. Days	
Experiment No.	BGS	SS
29A	40	28
29B	19	19
29C	52	48
30A	51	32
30B	91	80

Note on broths: in 10 of 12 columns of data for the above experiments, the Brilliant-Green Bile broth was the media of initial enrichment manure in batches as the ditch holding capacity is reached, regardless of loading rate. Thus, dissolved oxygen, B.O.D., pH,and total suspended solids may fluctuate drastically between emptying times. Others operate their channels for longer periods of time by allowing a lower loading rate by housing fewer cattle over the ditch and continually siphon off the effluent. Some units are improperly designed or misused. Because of these and the many other situations involving animal waste treatment, there continues to be excessive pollution of the environment with little control of contained and subsequently disposed zoonotic pathogens, including Salmonella.

As the livestock production industry becomes more familiar with this new method of treating and disposing of solid waste, and better guidelines develop to aid the operator, problems facing the producer today will be resolved. Systems will be adopted which will make the operation of an oxidation ditch less difficult. However, the aerobic process will remain and the waste will continue to contain pathogens which will survive the manure treatment, perhaps even grow and multiply within it.

Within the conduct of these experiments on the survival of Salmonella typhimurium the MPN (Most Probable Number) was not calculated. Nevertheless, since multiple tube methods of detection were used, the data validly indicated that S. typhimurium did not multiply in the manure of the model ditch, but rather declined in number with time. This statement is made on the basis of two criteria:

1. The proportionate number of samples positive deminished with time, and

2. They became more difficult to isolate with time, i.e., longer incubation times were required, and fewer colonies were found per plate. Although the most probable number was not done at any time, we are quite certain that the salmonella did not multiply, but rather followed a decimal reduction time which was shorter at summer temperatures (20C) than at winter temperatures (2C).

This finding is in accord with Rankin, et. al. (57) who did MPN and obtained similar results from animal waste slurry studies.

The current studies with Salmonella typhimurium utilized as a model organism revealed that this enteric pathogen survived in oxidation ditch conditions for 47 days, and in the field, could feasibly contaminate the environment once released into it. Thus, the interaction within the world of health would be altered and the effects far-ranging. The spread of drug-resistant pathogens and the ensueing transfer of this resistance which has developed from the treatment of livestock or the use of medicated feed (63, 64) are good examples of the type of present day and future problems with which we must cope. If this situation cannot be confined to the environment of the housing unit itself, the public health effects are sure to be felt throughout the entire livestock industry and its related environment. The world of health is an all-encompassing one in which we find man and his domestic animals co-existing with other living beings. Appropriate health practice must not be jeoprodized by contamination of our environment.

Perhaps one approach to controlling the salmonellae problem in animal manures is to chlorinate, or otherwise treat and disinfect the waste emanating from confinement housing units. However, with present methods, disinfection is not practical. Preliminary information from experiments conducted in the laboratory indicates that chlorination of oxidation ditch effluent destroys S. typhimurium. Due to the high organic content and the cost, disinfection of sludge, or manure itself, to eliminate pathogens would be extremely difficult. If chlorination or disinfection of feedlot, poultry lot, and other animal lot wastes were possible, it would be a meaningful measure of disease prevention and control. Additional research is required before guidelines can be established. Livestock do produce enormous amounts of manure waste, and the trend toward confinement housing and concentration of livestock makes this industry a tremendously important potential polluter if new methods and systems are not developed for handling and treatment of wastes. The increasing size of livestock production units in many instances creates public health problems of equal or greater magnitude than do municipal sewage plants which do not always destroy salmonellae (65,66).

ENGINEERING STUDIES OF OXIDATION DITCH OPERATION

One of the major problems with oxidation ditch operation is the inability to keep all solids in suspension. Mass accumulation of solids in any portion of the ditch promotes anaerobic conditions because of insufficient oxygen within the body mass. As stated earlier, the principal objective of the Model B studies was to determine and evaluate those factors affecting solid settlement patterns within the ditch. When it is impossible to eliminate the settlement of solids it may be desirable (or necessary) to remove them from the ditch for disposal by other means.

Solid Settlement Studies: Initial tests with the Model B oxidation ditch (Figures 6 and 7, see Introduction) were run using beef manure from the Rosemount field oxidation ditch as the solids material. It was found, however, that use of such wastes created extremely murky conditions making it difficult to observe and photograph the movement and settlement of the solids. A preliminary testing program was also made for proper selection of rotor design (Figure 2C).

For the above reasons a search of various organic and inorganic materials was made in an attempt to find a more suitable material to serve as the solids medium. Following extensive testing, small plastic spheres were found to be most suitable. Such spheres are commercially available in various diameters and specific gravities. The spheres used were chemically inert, of two diameters (1/8" and 3/32"), and of four specific gravities (0.91, 1.14, 1.17, and 1.30). Each type of sphere was color-coded, according to its particular size and specific gravity, thus permitting ready identification. Specific gravities were chosen so as to represent a range known to exist in typical beef animal manure wastes.

Unexpected problems of water quality were also encountered. Data obtained from certain early runs proved invalid because of a gradual incrustative condition which developed along the bottom and walls of the channel. Such incrustation, which occurred as a result of using tap water containing some 10 grains per gallon of hardness forming minerals, resulted in unpredictable roughness conditions within the channel. Demineralized water was then used in an effort to overcome the incrustation problem and was found to be satisfactory. Because of the relatively large amounts of air entrained in the demineralized water, however, small bubbles formed on the bottom of the channel and on a large percentage of the plastic spheres, causing them to move in a retarded and unatural fashion. The latter difficulty was overcome by allowing the water to stand overnight, thus de-aerating itself before a test was made.

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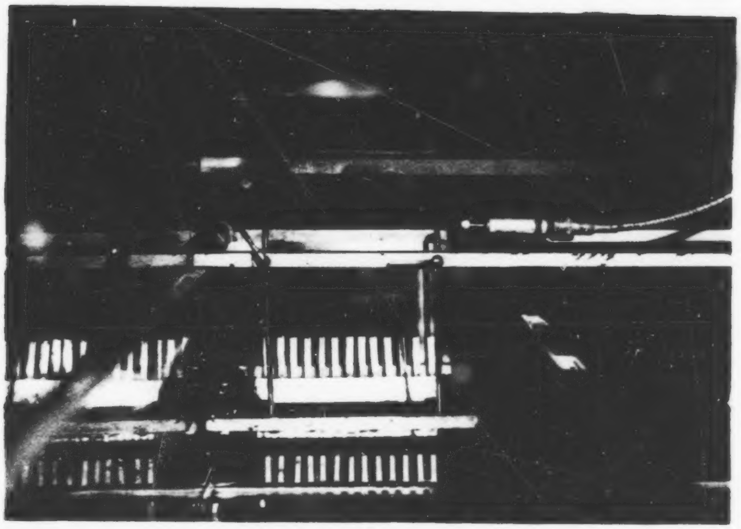

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Fig.2C Modified Rotor as Used in Model B Oxidation Ditch

Each solid-settlement test was run for a period of 15 minutes. Before the commencement of any given test the plastic spheres were dry-weighed and distributed evenly on the bed of the model ditch. The rotor, which had previously been positioned at one of three locations (A, B or E, as shown on page 72) was started. The rotor speed for all tests reported was 150 revolutions per minute. During the test period (15 minutes) the solids were forced to move within the ditch in a fashion as dictated by the particular test conditions, as enumerated in a fashion as dictated by the particular test conditions, as enumerated in Table 1. Following each test period the rotor was stopped and the percentage of solids found in the storage sump and beneath the rotor was determined.

Velocity Distribution Studies: Fluid flow in open channels is seldom uniform in nature. This nonuniformity is especially evident where curvilinear conditions are imposed, as at the ends of the typical oxidation ditch. Under such conditions, velocities vary widely both horizontally across the channel width as well as with water depth. The ability of a stream to transport solids depends to a large extent upon its velocity. Regions of a channel where low velocities exist, therefore, have less ability to suspend and carry solids than do regions of higher velocity. Depending upon the size and density of the solid, a critical velocity exists below which settlement of the solid will occur. Since in most oxidation ditch operations it is important to keep the finer particles in suspension at all times, the velocity must always equal or exceed the critical velocity for such solids.

This part of the study was aimed at the problem of velocity distribution in an oxidation ditch, and to determine those regions within the ditch having velocities sufficiently low to cause solid settlement. To measure low velocities, as they occur in such a hydraulic model, it became necessary to construct and calibrate a suitable velocity measuring insturment or device. After thorough investigation it was decided that a "tethered sphere" meter* was most suitable. Figures 3B and 4B show the basic construction of such a meter, which consists principally of a wax (or plastic) sphere suspended by a fine fiber (in this case a single strand of dental floss). When placed in a stream, the sphere is deflected from a vertical position in response to the magnitude of the velocity. In addition to its ability to measure low velocities, such a meter is capable of measuring point velocities in areas having

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*Original research on tethered sphere meters made by Dr. Henize Stefan and Frank R. Schiebe, and reported in Technical Paper No. 32, Series A, of the St. Anthony Falls Hydraulic Laboratory, University of Minnesota, Minneapolis, Minnesota.

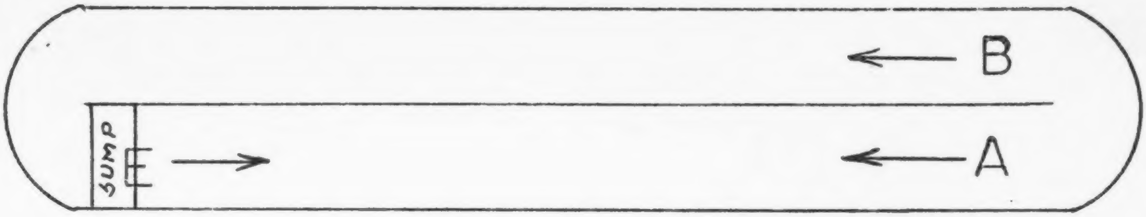


Figure 7B . Rotor Locations and Flow Directions Used in Solid Settlement Tests.

Table 1. Solid-Settlement Tests

Rotor Location	Water Depth	% Solids in Sump*	% Solids Under Rotor*	Rotor Vane
B	2"	29.3	51.5	in
B	2"	19.5	38.6	out
A	2"	36.1	38.8	in
A	2"	18.9	45.1	out
E	2"	31.5	----	in
E	2"	39.9	----	out
A	1 1/2"	35.4	37.0	in
A	1 1/2"	14.2	39.2	out
B	1 1/2"	45.4	49.5	in
B	1 1/2"	24.0	45.1	out
E	1 1/2"	52.6	----	in
E	1 1/2"	56.2	----	out
A	1"	35.6	48.9	in
A	1"	37.9	39.2	out
B	1"	69.8	22.1	in
B	1"	49.8	21.2	out
E	1"	87.1	----	in
E	1"	72.5	----	out

* Values shown represent average value of two or more runs for each condition.

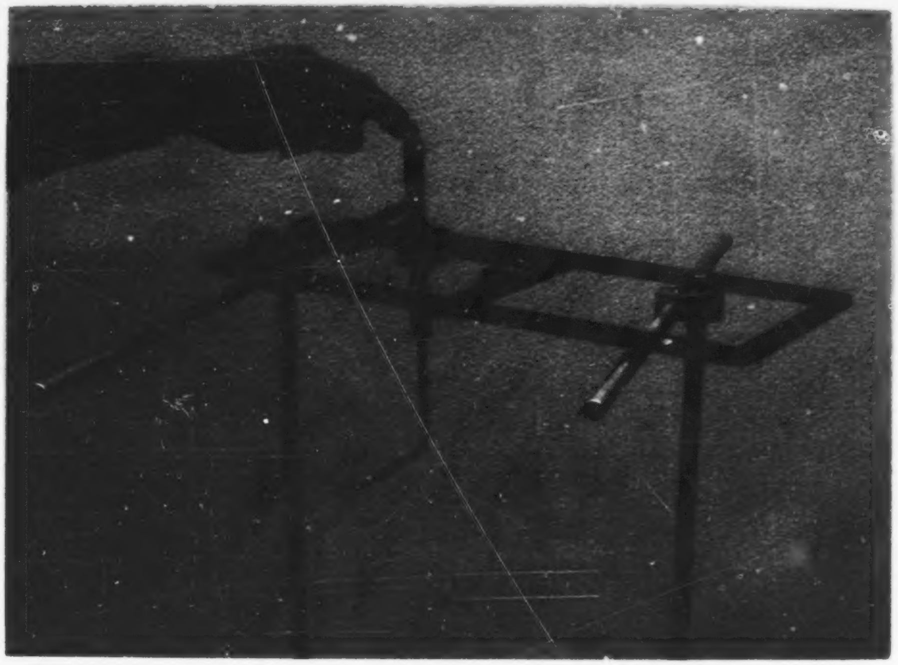
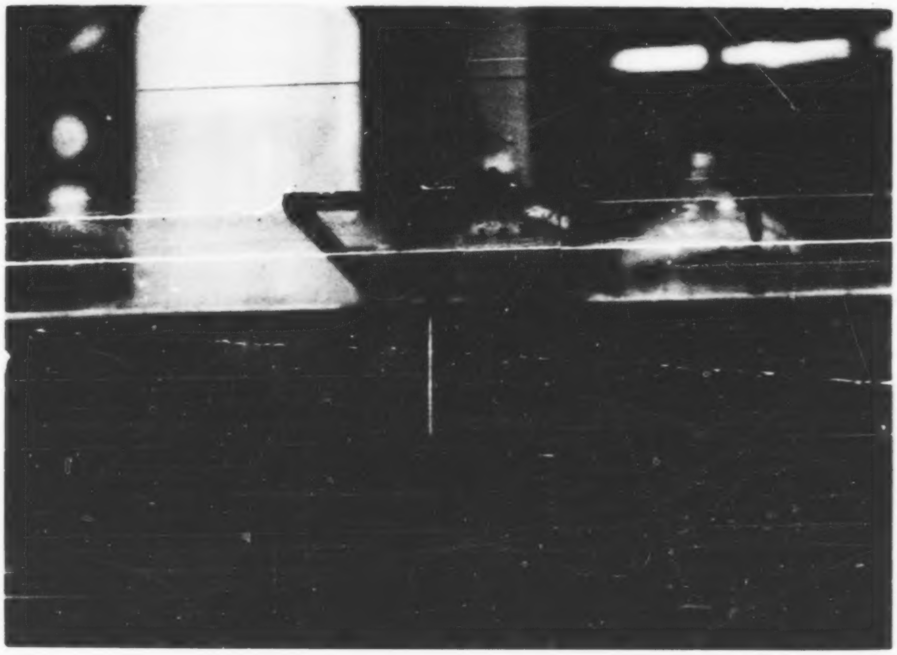


Figure 3B. Tethered-ball meter used to measure low velocities in Model B oxidation ditch.



Fibure 4B. Velocity meter as used to measure point velocities in oxidation ditch (side view).

severe space limitations.

Results

On the basis of the observations made in this study it is very difficult to prevent solids settlement and accumulation immediately beneath the rotor. As indicated earlier, such accumulations are undesirable since anaerobic conditions are likely to develop with subsequent odor problems. An inspection of the data shown in Table 1, together with Figures 5B and 6B shows that regardless of water depth in the ditch, solids did accumulate beneath the rotor. As to be expected, these data indicate that maximum separation of solids from the liquid occurs when the rotor is placed in a position directly above the collection sump. For water depths of 1", with rotor positioned above the sump, (Figure 7B) shows from 72 to 87 percent of the solids were collected in the sump.

In an effort to reduce the accumulation of solids beneath the rotor, a vane, shaped in the form of a Venturi throat-section, was designed and installed in the rotor vicinity of the ditch. Data shown in Figures 8B through 10B indicate that such a vane had an insignificant effect on the removal of solids from beneath the rotor. Whether vanes of these configurations might have a significant effect upon the accumulation of solids in other areas of the ditch was not determined.

The use of the throat-shaped vane in the vicinity of the rotor had significant effect upon percentage of solids collected in the sump, when the rotor was located at the A and B positions, (see Figures 11B and 12B). Again, however, the presence of the vane showed no effect with the rotor at the E position, as shown in Figure 10B.

Tables 2B through 19B (Appendix B) show velocity values as measured at 24 locations in the ditch. All velocities were measured at the one-inch depth. As noted, velocity distribution measurements were taken under varying conditions of water depth, rotor immersion depth, and rotor location. These velocity data were then plotted on scaled outlines of the ditch, Figures 13B through 30B (Appendix B) from which the velocity at any given point were determined. Shaded areas indicate those points or regions where reverse velocities occurred.

Inspection of the tabulated data, and of Figures 13B through 30B (Appendix B) shows that reverse velocity conditions occurred in many runs. The presence or absence of reversed flow conditions in a given area is of critical importance since surrounding such areas velocities must be at or near zero. Such low velocity conditions cause deposition of solids,

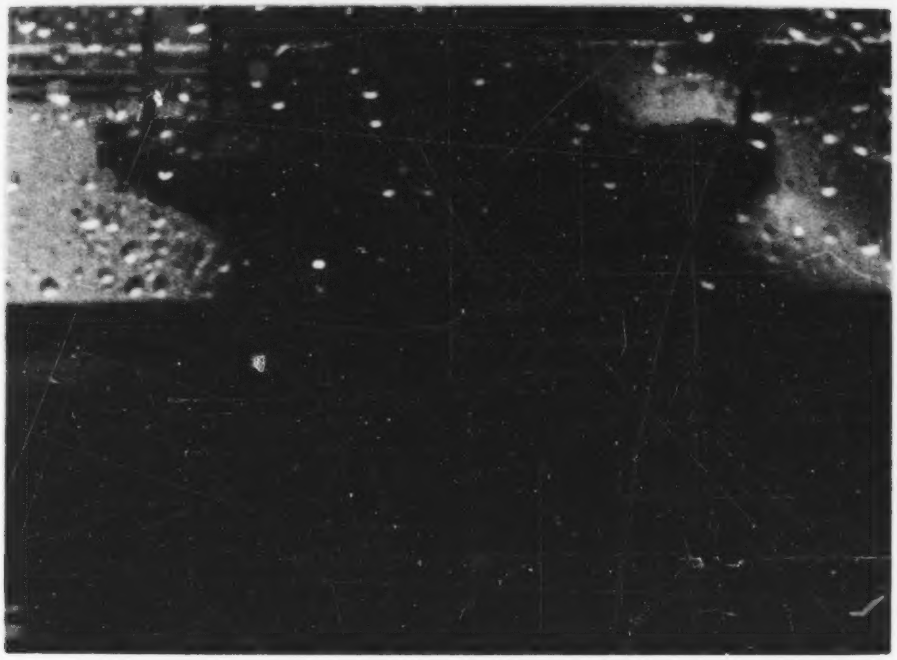


Figure 5B. Typical solids accumulation beneath rotor of oxidation ditch.

Figure 6B. Solids accumulation in low velocity areas in oxidation ditch (overhead view).

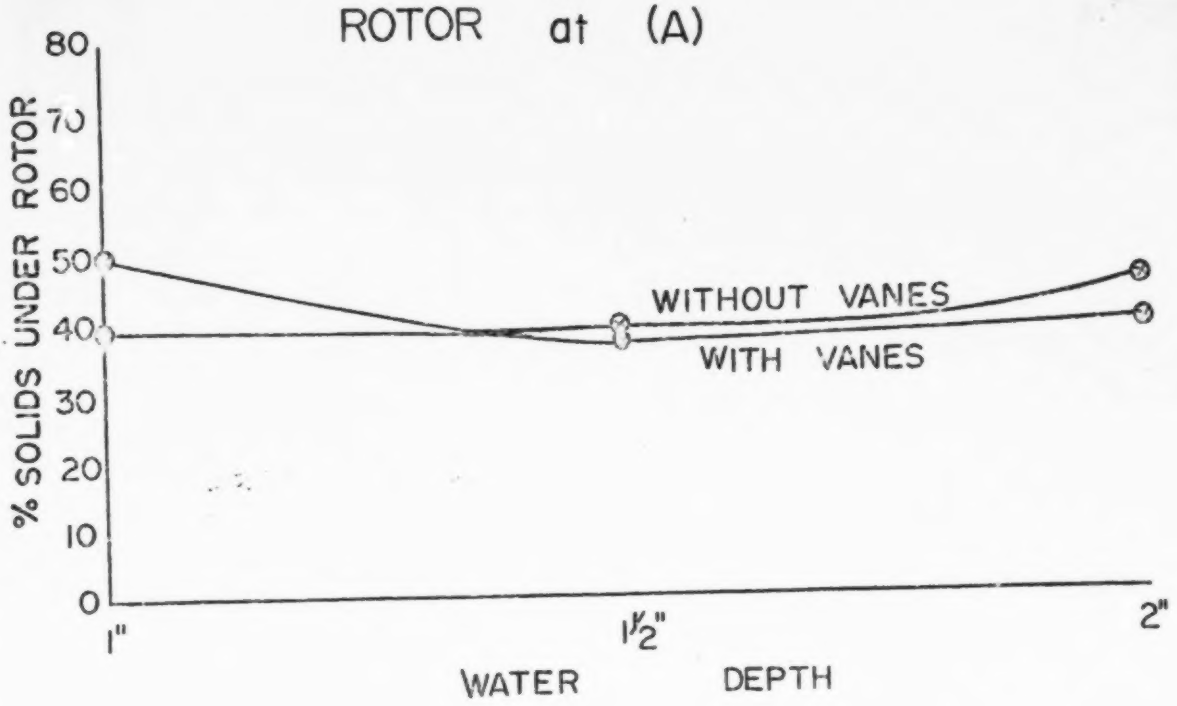


Figure 8B. Effect of Water Depth on Solid Settlement Beneath Rotor, with Rotor at Position A.

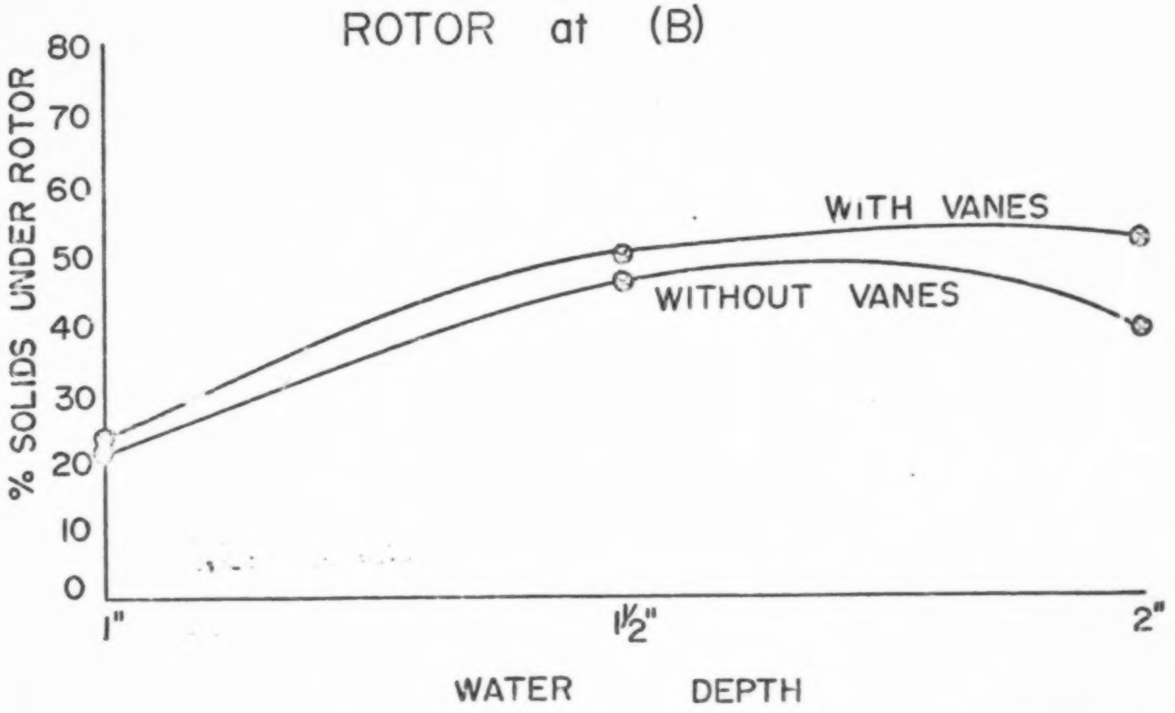


Figure 9B. Effect of Water Depth on Solid Settlement Beneath Rotor, with Rotor at Position B.

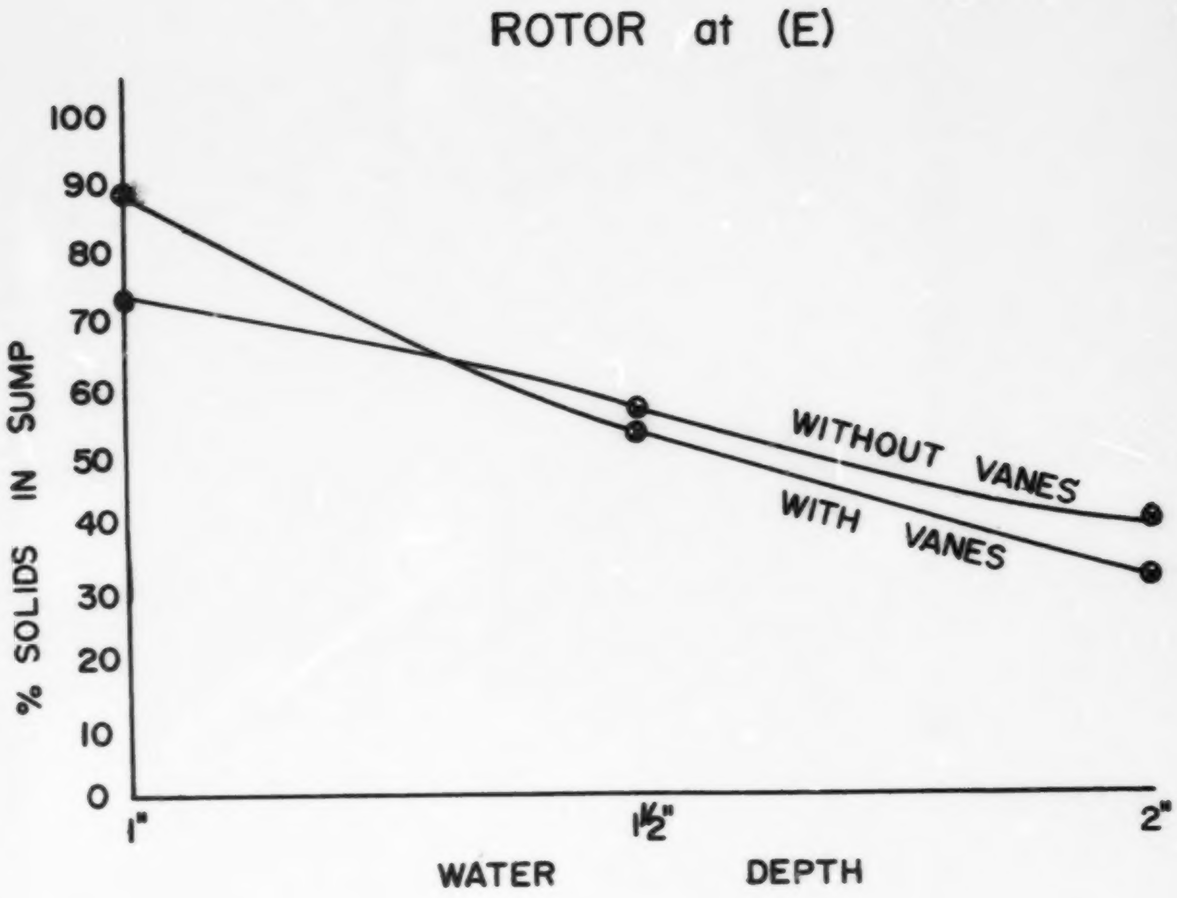


Figure 10B. Effect of Water Depth on Solid Settlement in Sump, with Rotor at Position E.

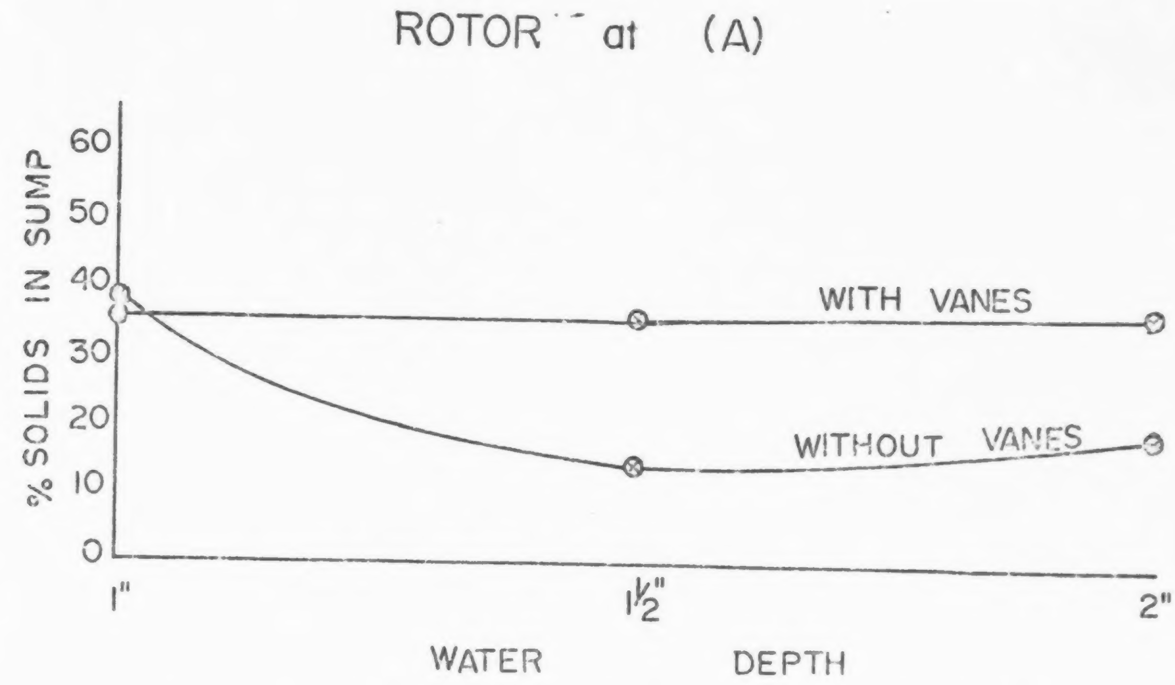


Figure 11B. Effect of Water Depth on Solid Settlement in Sump, with Rotor at Position A.

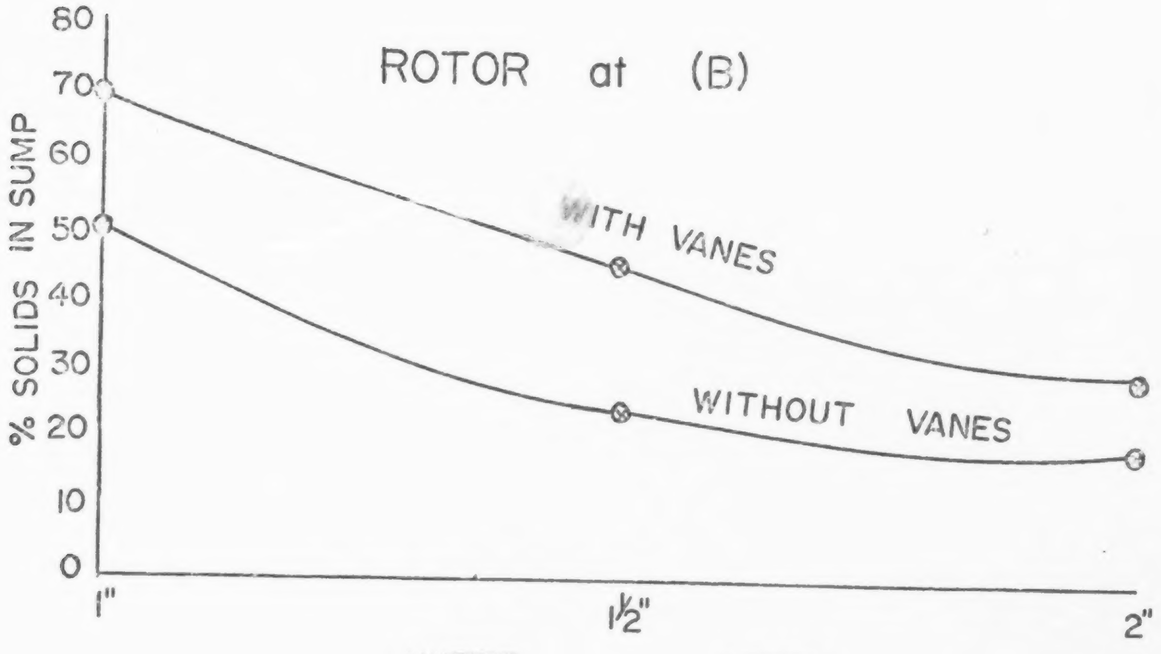


Figure 12B. Effect of Water Depth on Solid Settlement in Sump, with Rotor at Position B.

which as stated previously, may create partial clogging of the ditch, anaerobic decomposition and other undesirable problems. Observations of the shape and extent of the settled solids in low velocity regions provides some insight as to the corrections necessary for modifying the contour confirmation of the streambed.

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LEPTOSPIROSIS

APPENDIX-A

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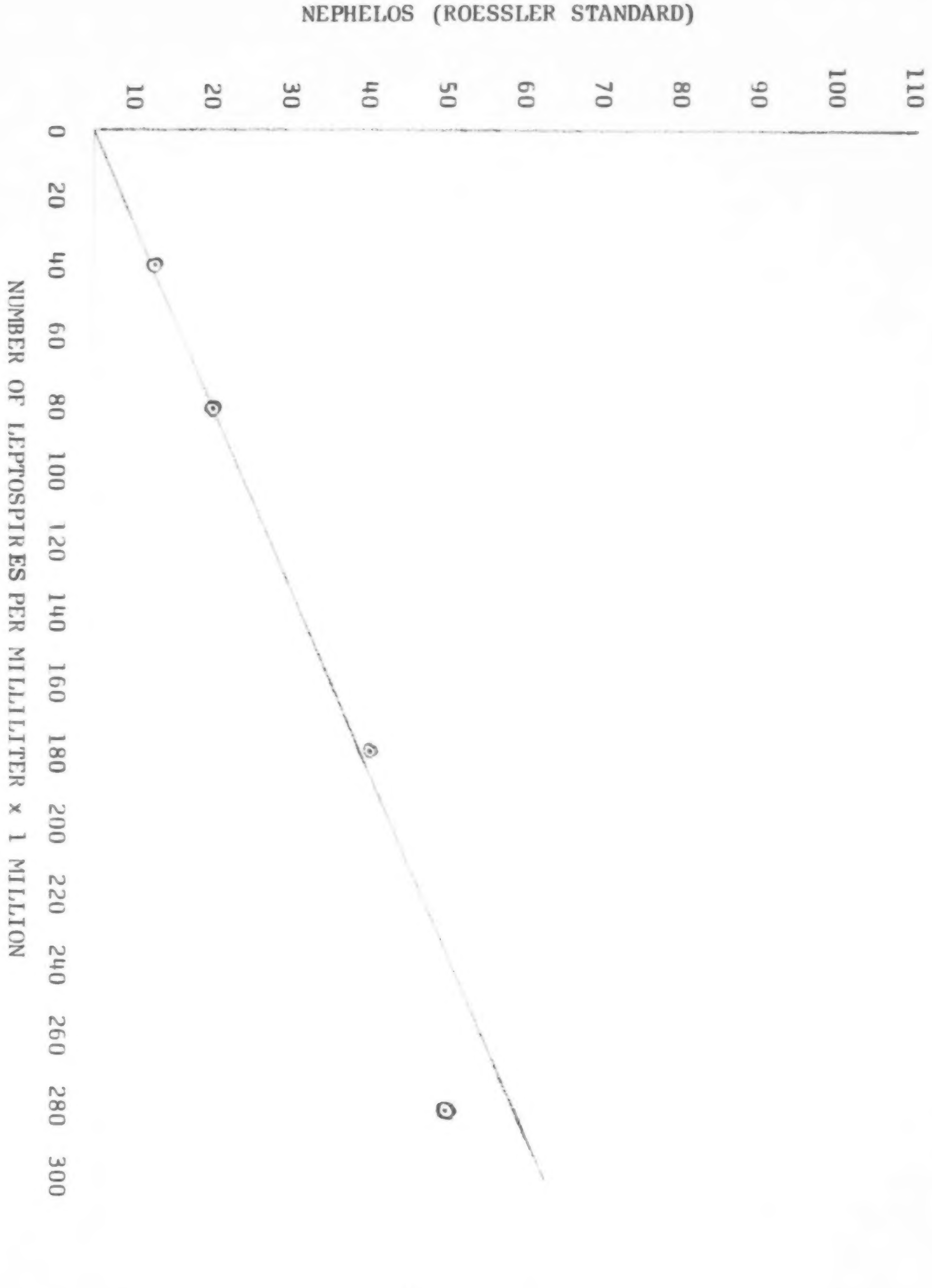
NEPHELOS (ROESSLER STANDARD)
10
20
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120
140
160
180
200
220
240
260
280
300
NUMBER OF LEPTOSPIRES PER MILLILITER x 1 MILLION

Fluorescent Antibody Methods Used in Leptospire Detection

A Zeiss FA microscope was used in the fluorescent antibody procedure, equipped with light source HBO Osram 200 W/4 (high intensity micro-scope illuminator), darkfield condensor, heat absorbing filter KG I, and exciter filters BG 38, and BG 12. *Leptospira pomona* fluorescein conjugate (Sylvan Co.) was diluted, 1 part with 9 parts PBS (phosphate buffered saline) and incubated with 0.1 gm bovine liver powder for 30 min. in 37C water bath with inversion every 5-10 min. The conjugate was then centrifuged at 3,000 rpm for 20 min. and the supernate drawn off and stored at -5C in 0.5 ml quantities.

The following controls were used during FA studies.

Slide 1. Known antigen *L. pomona* incubated with known *L. pomona* conjugate.

Slide 2. Known antigen *L. pomona* incubated with specific antiserum, rinsed briefly with PBS, and incubated with specific conjugate.

Slide 3. Known antigen *L. pomona* incubated with anti-brucella conjugate.

Slide 4. Sterile PBS and incubated with specific conjugate.

The following FA procedure was used to detect leptospires in manure.

Slides were pre-soaked in a 6% Tween 80. One drop of feces was spread over a 15mm^2 area and allowed to air dry, or dry with circulating heated air. The material was then fixed in acetone 5 minutes and dryed at 37C 5 minutes, followed by rinses in 3% Tween 80 in PBS 10 minutes and PBS 3 minutes. Excess moisture was removed and conjugate applied for 30 minutes at 37C in a moist chamber. The slides were rinsed in PBS and distilled water and dried. Cover slips were then mounted with elvanol or Difco mounting fluid and observed.

Agar Plate Technique for Isolating Leptospires

In order to substantiate that leptospires survive in animal manures the organisms must be isolated culturally. The abundance and broad spectrum of microorganisms in animal manure appeared to be an overwhelming demand for this research project entitled the Survival of Pathogens in Animal Manure Disposal. In 1957 Cox and Larson (38) described the first successful growth of leptospirae as isolated colonies, and many workers have continued to improve techniques or devise new methods in a quest for rapid, efficient, and pure isolation of the organisms.

The retrieval of serotype pomona from seeded beef cattle manure was essential to measure survival and to fulfill one objective of this research project.

Smibert (39) isolated leptospires by taking advantage of the selective penetration through Millipore filters, and Rittenberg, et. al. (40) illustrated the feasibility of Swinny filtering culture broth to isolate leptospires.

After much experimentation with previously described techniques, including the tube dilution schema, and manv attempts to culturally isolate leptospirae which could be observed with darkfield and Fluorescent Antibody microscropy, a method was developed which achieved the results hoped for.

Other bacterial growth was selectively restricted or inhibited pharmacologically with use of 5-fluorouracil (37). A dilution procedure was also used to advantage to isolate leptospirae from contaminated cultures.

By taking advantage of these previously mentioned techniques, we were able to develop a procedure with which we are able to isolate leptospires in pure culture from animal manure held at 20C.

Materials

1. Rabbit serum agar plates with 0.22 Millipore filters arranged as according to Smibert.
2. Liquid media: Bovine Serum Albumin according to Johnson (36).
3. Semi-solid: Bovine Serum Albumin-Agar according to Johnson(36).
4. Millipore Swinny hypodermic adapter fitted with millipore HA 0.45 m filter.

Method

1. Pipette approximately 5 ml seeded manure sample.

2. Place 3-5 drops of the material onto the center portion of the Millipore-curtain ring arrangement on the rabbit serum agar plates.

3. Incubate at 29C for 4 to 14 days (7 preferred) with the filters and ring in place. This prevents surface overgrowth by fungi which are present in the manure and the environment. It is important to maintain an ambient humidity of 80-90% to prevent drying of the plates.

4. After the 4-14 day period of incubation the filter and ring are removed.

5. A plug of plate media suspected of harboring leptospiral growth is crushed between a coverslip and glass slide and examined for leptospires by darkfield microscopy.

6. If leptospires are observed a plug of the agar media is removed with a loop and placed within the barrel of a 3 cc disposable syringe and the plunger replaced.

7. Approximately 1 cc of liquid medium may be aspirated into this syringe.

8. The material was forced through a 23 gauge needle into the barrel of a 5 cc glass syringe fitted with a Millipore Swinny hypodermic adapter containing a 0.45 filter.

9. The glass plunger was replaced and liquid medium was aspirated through the Swinny to make approximately 3-5 cc of material in the glass syringe.

10. This material was forced through the Swinny filter into a tube containing liquid medium.

11. A side-by-side series of 6 liquid and 6 semi-solid media was arranged in a rack and serial 10 fold dilutions and cultures made.

12. Incubate at 29C and examine according to media. Generally, we would darkfield examine the liquid cultures at 3-7 days and the semi-solid cultures at 5-14 days.

Laboratory Procedure Used to Determine Total Solids (TS)

Three (or more) porcelain evaporating dishes (50 to 100 ml. capacity) were placed for drying in a 103'- 105'C oven for at least 20 minutes. A measured quantity or sample of the liquid waste was poured into each evaporating dish and total weight determined. The evaporating dishes (with waste liquid) were placed on a steam table, where they remained until all visible water was evaporated. The dish and remaining solids residue were then transferred to 103' 105'C oven for overnight drying period. Dishes with dry residue then removed from oven, cooled in desiccator, and weighted.

Calculation:

$$\text{mg/l residue on evaporation} = \frac{\text{mg residue X 1,000}}{\text{ml sample}}$$

Laboratory Procedure Used to Determine Total Volatile Solids (TVS)

Dry residue samples obtained in (TS) procedure above ignited in electric furnace at 600'C to constant weight, usually requiring one hour. Loss on ignition reported as mg/l total volatile solids and the residue as mg/l fixed solids. Dishes allowed to cool briefly in air then cooled in desiccator. Although most TVS values exceeded 1,000 mg/l, calculations were reported to four significant figures.

$$\text{mg/l} = \frac{\text{mg (TS) residue - mg fixed solids}}{\text{ml sample}} \text{ X 1,000}$$

SURVIVAL AND DETECTION OF LEPTOSPIRES IN ANIMAL MANURE DISPOSAL

Day	Manure Added (lb)	Manure Environment				Barometer Mean (in.)	Survival and Detection Measurements								
		pH Mean	Ditch Temp. Mean (C)	D.O. Mean (ppm)	Total Solids (ppm)		Tubes Candle			Darkfield Candle			PA Candle		
							A	B	C	A	B	C	A	B	C
Experiment No. 1 LW Candle Studies at Winter Temperatures															
1		9.2	3.5	10.1		30.30	ND	ND	ND	ND	ND	ND	ND	ND	ND
2		9.2	4.0	13.6	12,239	30.33	TP	-	T	++	+	+	+	+	+
3		9.2	3.5	14.2		30.27	-	-	-	+	+	+	+	+	+
4		9.3	3.0	14.2		ND	-	-	-	+	+	+	+	-	+
5		9.3	3.0	13.9		ND	-	-	-	+	+	+	+	?	?
6		9.3	3.0	13.5		ND	-	-	-	+	+	+	+	?	?
7		9.3	2.5	13.5	8,414	ND	-	-	-	+	+	+	+	?	+
8		9.2	2.0	13.6		30.26	-	-	-	+	+	+	+	+	+
9		9.2	1.5	13.8		30.25	-	-	-	+	+	+	+	+	+
Mean		9.2	2.0	13.4		30.29									
Experiment No. 2 LW Candle Studies at Winter Temperatures															
1		9.2	2.0	13.2	7,960	29.81	ND	ND	ND	++	++	++	ND	ND	ND
2		9.2	1.0	13.0		30.14	T	-	T	+	+	+	+	+	+
3		9.1	1.0	13.2	7,731	29.96	-	-	-	++	+	+	+	+	+
4		8.9	1.7	13.5		30.08	-	-	-	++	+	+	ND	ND	ND
5		8.9	2.0	13.7		ND	-	-	-	+	+	+	ND	ND	ND
6		8.9	2.0	13.7		ND	-	-	-	+	+	+	ND	ND	ND
7		8.9	2.0	13.3		30.42	-	-	-	+	+	+	ND	ND	ND
8		8.9	1.0	12.3		29.75	-	-	-	+	+	+	ND	ND	ND
9		8.9	3.5	12.0		30.23	-	-	-	+	+	+	ND	ND	ND
10		8.9	4.0	12.0		30.06	-	-	-	+	+	+	+	+	+
Mean		9.0	1.7	13.4		30.06									

SURVIVAL AND DETECTION OF LEPTOSPIRES IN ANIMAL MANURE DISPOSAL

Day	Manure Added (lb)	Manure Environment				Barometer Mean (in.)	Survival and Detection Measurements								
		pH Mean	Ditch Temp. Mean (C)	D.O. Mean (ppm)	Total Solids (ppm)		Tubes Candle			Darkfield Candle			PA Candle		
							A	B	C	A	B	C	A	B	C
Experiment No. 3 LW Candle Studies at Winter Temperatures															
1		9.0	2.0	12.5	6,748	30.22	ND	ND	ND	ND	ND	ND	ND	ND	ND
2		9.0	2.0	12.4		30.20	-	-	-	++	++	++	+	+	+
3		9.0	2.5	12.9		30.06	-	-	-	+	+	+	ND	ND	ND
4		9.0	2.0	13.0		29.96	-	-	-	+	+	+	ND	ND	ND
5		9.0	2.7	12.7	6,596	30.05	-	-	-	+	+	+	ND	ND	ND
6		9.0	2.0	12.5		29.87	-	-	-	+	+	+	ND	ND	ND
7		9.0	2.0	12.0		30.00	-	-	-	+	+	+	ND	ND	ND
8		9.0	2.0	13.0	6,268	30.17	-	-	-	+	+	+	ND	ND	ND
9		9.0	3.0	13.4		30.12	-	-	-	+	+	+	+	+	+
Mean		9.0	2.3	12.7		30.07									
Experiment No. 4 LW Candle Studies at Winter Temperatures															
1		8.4	2.0	5.9	9,511	30.14	ND	ND	ND	ND	ND	ND	ND	ND	ND
2		8.4	2.0	8.4		29.98	-	-	-	++	++	++	+	+	+
3		8.5	2.0	8.1		30.08	-	-	-	++	++	+	ND	ND	ND
4		8.5	2.0	7.3	9,913	29.99	-	-	-	+	+	+	ND	ND	ND
5		8.5	2.0	7.2		29.06	-	-	-	+	+	+	ND	ND	ND
6		8.5	2.0	7.0		29.31	-	-	-	+	+	+	ND	ND	ND
7		8.4	2.0	7.8		29.32	-	-	-	+	+	+	ND	ND	ND
8		8.5	2.0	7.9	9,643	29.94	-	-	-	+	+	+	ND	ND	ND
9		8.3	2.5	7.0		29.94	-	-	T	+	+	+	ND	ND	ND
10		8.3	2.0	6.8		29.47	-	-	-	+	+	+	+	+	+
Mean		8.4	2.0	7.3		29.72									

SURVIVAL AND DETECTION OF LEPTOSPIRES IN ANIMAL MANURE DISPOSAL

Day	Manure Added (lb)	Manure Environment				Barometer Mean (in.)	Survival and Detection Measurements								
		pH Mean	Ditch Temp. Mean (C)	D.O. Mean (ppm)	Total Solids (ppm)		Tubes Candle			Darkfield Candle			PA Candle		
							A	B	C	A	B	C	A	B	C
Experiment No. 5 LW Candle Studies at Winter Temperatures															
1	2.2	8.2	2.3	6.7	9,480	29.67	ND	ND	ND	ND	ND	ND	ND	ND	ND
2	2.2	8.3	3.0	6.7		29.48	T	-	T	+	+	+	+	+	+
3		8.5	2.0	6.9		29.07	-	-	T	+	+	+	ND	ND	ND
4		8.2	2.0	7.3	8,596	29.58	-	-	T	++	+	+	ND	ND	ND
5	2.2	8.2	3.0	6.0		29.71	-	-	T	+	+	+	ND	ND	ND
6	2.2	8.5	3.0	5.9		29.37	-	-	T	+	+	+	ND	ND	ND
7	2.2	8.5	3.1	6.0	8,425	29.60	-	-	T	+	+	+	ND	ND	ND
8	2.2	8.4	3.3	5.9		29.75	-	-	T	+	+	+	+	+	+
Mean		8.4	2.7	6.4		29.53									
Experiment No. 6 LW Candle Studies at Winter Temperatures															
1	2.2	8.4	3.0	5.4	8,126	29.95	ND	ND	ND	ND	ND	ND	ND	ND	ND
2	2.2	8.3	4.0	5.7		29.83	-	-	T	++	++	++	+	+	+
3		8.2	2.8	5.8		29.84	T	-	T	+	+	+	+	+	+
4	2.2	8.1	2.8	5.6	8,434	29.92	T	-	T	++	+	+	ND	ND	ND
5	2.2	8.2	2.3	5.4		29.93	-	-	-	++	+	+	ND	ND	ND
6		8.3	2.0	5.6		29.87	-	-	T	+	+	+	ND	ND	ND
7		8.2	2.0	5.3		ND	-	-	-	+	+	+	ND	ND	ND
8	2.2	8.2	2.0	5.4		29.80	-	-	T	+	+	+	ND	ND	ND
9	2.2	8.2	2.0	5.1		29.45	-	-	T	+	+	+	ND	ND	ND
10	2.2	8.2	2.0	5.3		29.48	-	-	T	+	+	+	ND	ND	ND
11		8.1	2.0	5.4	9,045	29.85	-	-	T	+	+	+	ND	ND	ND
12		8.2	2.0	5.4		29.84	-	-	T	+	+	+	+	+	+
Mean		8.2	2.4	5.43											

SURVIVAL AND DETECTION OF LEPTOSPIRES IN ANIMAL MANURE DISPOSAL

Day	Manure Added (lb)	Manure Environment				Barometer Mean (in.)	Survival and Detection Measurements								
		pH Mean	Ditch Temp. Mean (C)	D.O. Mean (ppm)	Total Solids (ppm)		Tubes Candle A	B	C	Darkfield Candle A	B	C	PA Candle A	B	C
Experiment No. 7 LW Effluent and Sludge Studies at Winter Temperatures															
1		8.8	4.3	4.2			ND	ND	ND	ND	ND	ND	ND	ND	ND
2		8.7	5.0	3.5			-	-	-	+	+	+	+	+	+
3		8.7	3.3	3.5			-	-	-	+	+	+	ND	ND	ND
4		8.7	3.3	2.6			-	-	-	-	+	-	ND	ND	ND
5		8.7	3.5	3.6			-	-	-	-	-	-	ND	ND	ND
6		8.7	2.8	3.5			-	-	-	+	+	-	ND	ND	ND
7		8.7	3.2	3.6			-	-	-	+	+	+	ND	ND	ND
8		8.5	2.8	3.5			T	-	-	+	+	-	ND	ND	ND
9		8.6	2.8	3.5			T	-	T	+	-	-	ND	ND	ND
10							T	T	-	+	-	+	+	+	+
Mean		8.7	3.4	3.5											
Experiment No. 8 LW Effluent and Sludge Studies at Winter Temperatures															
1		8.0	3.0	7.3			ND	ND	ND	ND	ND	ND	ND	ND	ND
2		3.5	3.5	2.7			-	-	-	+	+	+	+	+	+
3		8.5	2.5	2.5			-	T	-	+	+	+	ND	ND	ND
4		8.4	2.0	2.6			-	T	-	+	+	+	ND	ND	ND
5		8.3	2.6	5.5			-	-	T	+	+	+	ND	ND	ND
6		8.4	3.5	5.6			T	T	-	+	+	+	ND	ND	ND
7		8.2	3.0	5.4			T	-	-	+	+	+	ND	ND	ND
8		8.2	3.0	5.3			T	T	T	+	+	+	+	+	+
Mean		8.3	2.9	4.6											

SURVIVAL AND DETECTION OF LEPTOSPIRES IN ANIMAL MANURE DISPOSAL

Day	Manure Added (lb)	Manure Environment				Barometer Mean (in.)	Survival and Detection Measurements								
		pH Mean	Ditch Temp. Mean (C)	D.O. Mean (ppm)	Total Solids (ppm)		Tubes Candle A	Tubes Candle B	Tubes Candle C	Darkfield Candle A	Darkfield Candle B	Darkfield Candle C	PA Candle A	PA Candle B	PA Candle C
Experiment No. 9 LW Effluent and Sludge Studies at Winter Temperatures															
1		8.3	3.3	7.1			-	-	-	-	-	-	-	-	-
2		8.2	3.0	6.8			+	+	+	+	+	+	+	+	+
3		8.2	2.8	6.1			+	+	-	+	+	+	-	-	-
4		8.1	2.9	6.5			-	+	+	+	+	+	ND	ND	ND
5		8.4	3.0	7.8			+	+	+	+	+	+	ND	ND	ND
6		8.4	2.4	6.5			+	-	-	+	+	+	ND	ND	ND
7		8.4	2.6	7.0			+	-	-	+	+	+	ND	ND	ND
8		8.3	2.6	7.0			-	+	+	+	+	+	ND	ND	ND
9		8.2	2.5	6.9			+	+	-	+	+	+	ND	ND	ND
10		8.0	2.5	6.8			+	+	+	+	+	-	ND	ND	ND
11		7.9	2.5	6.5			+	+	+	+	+	+	ND	ND	ND
12		ND	ND	ND			+	+	+	+	+	+	+	+	+
Mean		8.2	2.7	6.8											

SURVIVAL AND DETECTION OF LEPTOSPIRES IN ANIMAL MANURE DISPOSAL

Day	Manure Added (lb)	Manure Environment				Barometer Mean (in.)	Survival and Detection Measurements					
		pH Mean	Ditch Temp. Mean (C)	D.O. Mean (ppm)	Total Solids (ppm)		Tubes		Darkfield		FA	
							X	Y	X	Y	X	Y
Experiment No. 10 LW Studies of Seeded Ditch at Winter Temperatures												
1		7.6	2.0	5.7	9,621	29.76	ND	ND	ND	ND	ND	ND
2		7.5	2.0	5.5		29.64	+	+	+	++	-	-
3		7.3	3.0	5.9		29.80	+	-	+	++	-	-
4		7.1	3.3	5.9	9,653	29.81	-	+	+	+	ND	ND
5		6.9	3.0	6.6		29.69	+	+	-	+	ND	ND
6	2.2	6.9	3.0	6.8	9,625	29.68	+	+	++	+	-	-
7	2.2	7.0	3.0	6.1		29.93	+	+	++	+	ND	ND
8	2.2	7.2	3.0	8.4		29.97	+	+	++	-	ND	ND
9	2.2	6.8	3.0	8.7	10,700	30.07	-	+	++	-	ND	ND
10		6.7	3.0	8.8		29.92	+	+	-	-	-	-
11		6.7	3.0	8.9		29.82	-	-	-	+	-	-
12		6.7	3.0	8.7		30.03	-	-	-	-	+	-
13		6.7	3.0	8.6	10,542	30.19	+	-	+	-	ND	ND
14		6.7	3.0	8.6		30.08	-	-	-	-	+	-
15		6.7	3.0	8.2		30.13	-	-	+	-	ND	ND
16	4.3	6.6	3.3	8.4	10,752	30.05	-	+	+	-	ND	ND
17	4.4	6.8	4.0	9.1		29.74	-	-	++	-	ND	ND
18		6.8	4.0	9.1		29.75	-	-	+	-	ND	ND
19		7.0	4.0	7.8		29.62	-	-	+	-	ND	ND
20		6.9	3.0	6.7		29.60	ND	ND	-	-	+	-
21		6.8	3.0	6.9		29.60	ND	ND	-	-	+	+
22		6.7	3.0	7.4		29.72	-	+	+	-	+	-
23		6.6	4.0	7.9		29.67	+	+	+	-	+	+
24		6.6	4.0	8.2		29.94	-	-	-	+	+	+
25		6.6	3.0	8.3		29.95	-	-	+	+	+	+
26		6.6	3.0	8.6		29.70	ND	ND	+	+	-	-
27		6.6	3.3	9.2		29.86	-	-	-	-	-	-
Mean		6.9	3.1	7.0		29.84						

++=motility

SURVIVAL AND DETECTION OF LEPTOSPIRES IN ANIMAL MANURE DISPOSAL

Day	Manure Added (lb)	Manure Environment				Barometer Mean (in.)	Survival and Detection Measurements								
		pH Mean	Ditch Temp. Mean (C)	D.O. Mean (ppm)	Total Solids (ppm)		Tubes Candle			Darkfield Candle			PA Candle		
							A	B	C	A	B	C	A	B	C
Experiment No. 1 LS Candle Studies at Summer Temperatures															
1	2.2	8.0	25.5	ND		ND	-	-	-	++	++	++	ND	ND	ND
2	2.2	8.2	25.8				-	-	-	++	++	++	ND	ND	ND
3	2.2	8.3	25.3				-	-	-	++	+	++	ND	ND	ND
4	2.2	8.5	24.1				-	-	-	+	-	+	ND	ND	ND
5	2.2	8.5	24.6				-	-	-	+	-	+	ND	ND	ND
6	2.2	8.5	24.0				-	-	-	+	-	+	ND	ND	ND
7	2.2	8.5	22.8				-	-	-	+	-	+	ND	ND	ND
8	2.2	8.5	23.2				-	-	-	+	-	+	ND	ND	ND
9	2.2	8.4	23.5				-	-	-	+	-	+	ND	ND	ND
10	2.2	8.3	24.3				-	-	-	+	-	+	ND	ND	ND
11	2.2	8.3	24.5				-	-	-	+	-	+	ND	ND	ND
12	2.2	8.4	24.4				-	-	-	+	-	+	ND	ND	ND
13	2.2	8.5	23.7		6,230		-	-	-	+	-	+	ND	ND	ND
14	2.2	8.3	24.7				-	-	-	+	-	+	ND	ND	ND
Mean		8.36	24.29												
Experiment No. 2 LS Candle Studies at Summer Temperatures															
1	2.2	8.5	19.6	0.5	20,906	ND	ND	ND	ND	++	++	++	+	+	+
2		8.5	20.2	0.6		29.32	-	-	-	++	+	+	+	+	+
3		8.4	18.7	0.5	14,446	ND	-	-	-	+	+	+	+	+	+
4		8.5	18.7	0.4	12,709	29.99	-	-	-	+	+	+	+	+	+
5		8.4	19.0	ND		ND	-	-	-	+	+	+	ND	ND	ND
6		8.5	19.0	3.4		ND	-	-	-	+	-	-	ND	ND	ND
7		8.3	20.0	1.1	6,268	ND	-	-	-	-	-	-	ND	ND	ND
Mean		8.44	19.31	1.08		29.66									

SURVIVAL AND DETECTION OF LEPTOSPIRES IN ANIMAL MANURE DISPOSAL

Day	Manure Added (lb)	Manure Environment				Barometer Mean (in.)	Survival and Detection Measurements								
		pH Mean	Ditch Temp. Mean (C)	D.O. Mean (ppm)	Total Solids (ppm)		Tubes Candle			Darkfield Candle			PA Candle		
							A	B	C	A	B	C	A	B	C
Experiment No. 3 LS Candle Studies at Summer Temperatures															
1		8.4	18.8	4.4	5,009	29.95	TP*	TP*	TP*	++	++	++	+	+	+
2	2.2	8.4	18.6	2.8		29.10	TP*	TP*	TP*	++	++	++	+	+	+
3	2.2	8.5	18.8	2.4	4,749	30.19	+	+	+	++	++	++	ND	ND	ND
4	2.2	8.7	19.0	3.0		29.70	+	-	-	++	++	++	ND	ND	ND
5	2.2	8.8	19.2	2.0		ND	+	-	-	++	++	+	ND	ND	ND
6	2.2	8.9	19.0	3.0		ND	-	-	-	ND	ND	ND	ND	ND	ND
7	2.2	8.7	18.0	2.5	5,106	30.20	-	-	-	+	+	+	ND	ND	ND
8	2.2	8.6	18.5	3.3		29.50	ND	ND	ND	+	+	+	ND	ND	ND
9	2.2	8.6	19.6	3.7		29.57	ND	ND	ND	+	+	+	ND	ND	ND
10	2.2	8.6	19.6	3.9	6,092	ND	ND	ND	ND	++	+	+	ND	ND	ND
11	2.2	8.5	20.4	0.4		ND	ND	ND	ND	+	+	+	ND	ND	ND
12	2.2	8.6	21.1	ND		ND	ND	ND	ND	+	+	+	ND	ND	ND
13	2.2	8.6	19.6	ND		ND	ND	ND	ND	+	+	-	ND	ND	ND
14	2.2	8.6	18.9	ND		ND	ND	ND	ND	+	+	-	+	-	+
Mean		8.61	19.22	2.84		29.77									

*Measurement of survival every 3 hours during first and second day.

++ = Motility
T = Tube
P = Plate

SURVIVAL AND DETECTION OF LEPTOSPIRES IN ANIMAL MANURE DISPOSAL

Day	Manure Added (lb)	Manure Environment				Barom-eter Mean (in.)	Survival and Detection Measurements								
		pH Mean	Ditch Temp. Mean (C)	D.O. Mean (ppm)	Total Solids (ppm)		Tubes Candle			Darkfield Candle			PA Candle		
							A	B	C	A	B	C	A	B	C
Experiment No. 5 LS Candle Studies at Summer Temperatures															
1	2.2	7.0	19.4	4.3		30.14	ND	ND	ND	++	++	++	ND	ND	ND
2		7.1	18.6	3.0	8,183	30.41	TP	TP	TP	++	++	++	+	+	+
3		7.0	19.0	5.7		30.02	P	T	-	++	++	++	ND	ND	ND
4		6.9	18.9	6.4		ND	T	T	T	++	+	+	ND	ND	ND
5		6.9	19.0	6.5		ND	P	-	-	++	+	+	ND	ND	ND
6	2.2	7.0	19.0	7.7	8,263	29.80	TP	T	T	++	++	+	ND	ND	ND
7	2.2	7.0	18.5	6.4		29.62	TP	T	T	++	++	+	ND	ND	ND
8	2.2	6.9	18.9	3.3		30.19	-	-	-	+	+	+	ND	ND	ND
9	2.2	6.9	18.3	4.4		29.75	-	-	-	+	+	+	ND	ND	ND
10	2.2	6.9	18.3	2.1	9,336	29.84	-	-	-	++	+	+	ND	ND	ND
11	2.2	6.9	19.0	1.7		ND	-	-	-	+	+	+	ND	ND	ND
12	2.2	7.1	19.0	4.0		ND	-	-	-	+	+	+	+	+	+
13		7.1	18.8	6.4		29.91	-	-	-	+	+	+	+	+	+
14		7.0	18.8	7.1		29.39	-	-	-	+	+	+	+	+	-
Mean		7.0	18.8	4.9		29.91									
Experiment No. 8A Candle Studies at Summer Temperatures															
1	2.2	8.2	20.0	4.9	5,761	30.10	ND	ND	ND	++	++	++	+	+	+
2	2.2	7.9	19.3	4.0		29.91	TP	TP	TP	++	++	++	+	+	+
3	2.2	8.0	18.7	6.6		ND	P	-	-	++	+	+	ND	ND	ND
4	2.2	8.4	19.7	5.9		30.22	TP	-	-	++	+	+	ND	ND	ND
5		7.9	19.3	1.8	6,012	30.22	-	-	-	++	+	+	ND	ND	ND
6		7.8	18.9	3.7		30.18	-	-	-	++	+	+	ND	ND	ND
7		7.8	19.6	3.4	6,053	30.18	-	-	-	+	+	-	ND	ND	ND
8		7.7	19.8	2.0		ND	-	-	-	+	+	-	ND	ND	ND
9		7.7	19.5	3.9		ND	-	-	-	+	+	-	+	+	-
Mean		7.88	19.37	3.96		30.14									

SURVIVAL AND DETECTION OF LEPTOSPIRES IN ANIMAL MANURE DISPOSAL

Day	Manure Added (lb)	Manure Environment				Barometer Mean (in.)	Survival and Detection Measurements								
		pH Mean	Ditch Temp. Mean (C)	D.O. Mean (ppm)	Total Solids (ppm)		Tubes			Darkfield			PA		
							T	M	B	T	M	B	T	M	B
Experiment No. 6 LS Effluent and Sludge Studies at Summer Temperatures															
1		ND	ND	ND			ND			++	++	++	+	+	+
2										+	++	++	+	+	+
3										+	++	++	+	+	+
4										++	++	++	+	+	+
5										++	+	-	+	+	+
6										+	++	-	+	+	+
7			Temperature at	20°C						++	++	-	+	+	+
8										+	++	-	+	+	+
9										-	+	-	+	+	+
Experiment No. 7 LS Effluent and Sludge Studies at Summer Temperatures															
1		9.1	19.0	10.0		ND	ND	ND	ND	+	+	+	ND	ND	ND
2		9.0	19.0	4.2			-	-	-	+	+	+	+	+	+
3		8.7	19.5	8.4			T	T	-	+	+	+	+	+	+
4		8.5	19.5	8.1			-	-	-	+	+	+	+	+	+
5		8.3	19.0	8.0			-	-	-	+	+	+	+	+	+
Mean		8.71	19.2	7.7											
Experiment No. 8 LS Effluent and Sludge Studies at Summer Temperatures															
1		8.5	18.0	8.3		ND	-	-	-	+	++	++	+	+	+
2		9.2	19.0	9.1			-	-	-	+	+	+	+	+	+
3		9.2	19.0	9.6			-	-	-	+	+	+	+	+	-
4		9.0	19.0	9.2			-	-	-	+	+	+	+	+	-
5		8.9	19.0	8.6			T	T	T	+	+	-	-	-	-
6		8.6	19.0	8.5			T	T	-	-	+	-	-	-	-
Mean		9.0	18.8	8.9											

SURVIVAL AND DETECTION OF LEPTOSPIRES IN ANIMAL MANURE DISPOSAL

Day	Manure Added (lb)	Manure Environment				Barometer Mean (in.)	Survival and Detection Measurements								
		pH Mean	Ditch Temp. Mean (C)	D.O. Mean (ppm)	Total Solids (ppm)		Tubes			Darkfield			PA		
							T	M	B	T	M	B	T	M	B
Experiment No. 9 LS Effluent and Sludge Studies at Summer Temperatures															
1							-	-	-	++	++	+	+	+	+
2							-	-	-	++	+	+	+	+	+
3				Effluent at 20°			-	-	T	+	+	-	+	-	-
4			No External Data Made				-	-	-	+	-	-	+	-	-
5							-	-	-	+	-	-	+	+	-
6							-	-	-	-	-	-	+	+	-
7							-	-	-	+	-	-	+	+	-
Experiment No. 10 LS Effluent and Sludge Studies at Summer Temperatures															
1		8.9	19.5	8.5			ND	ND	ND	+	+	+	ND	ND	ND
2		8.5	19.5	8.3			-	-	-	+	+	+	+	+	+
3		8.4	19.0	9.7		ND	-	-	-	+	+	+	ND	ND	ND
4		8.4	19.0	9.0			-	-	-	+	+	-	ND	ND	ND
5		8.4	18.5	9.8			-	-	-	+	-	-	ND	ND	ND
6		8.4	18.0	10.0			-	-	-	+	-	-	ND	ND	ND
7		8.4	17.5	10.2			-	-	-	+	-	-	ND	ND	ND
8		8.4	19.5	8.5			-	-	-	+	-	-	+	+	+
Mean		8.5	18.8	9.2											

SURVIVAL AND DETECTION OF LEPTOSPIRES IN ANIMAL MANURE DISPOSAL

Day	Manure Added (lb)	Manure Environment				Barometer Mean (in.)	Survival and Detection Measurements					
		pH Mean	Ditch Temp. Mean (C)	D.O. Mean (ppm)	Total Solids (ppm)		Tubes--		Darkfield		PA	
							X	Y	X	Y	X	Y
Experiment No. 11 LS Studies of Seeded Ditch at Summer Temperatures												
1		8.2	18.7	4.1	4,902	29.74	ND	ND	ND	ND	ND	ND
2		8.2	19.4	3.4		29.72	ND	ND	ND	ND	ND	ND
3		8.2	18.4	3.2		29.66	-	-	-	-	ND	ND
4		8.2	17.0	2.6		ND	-	-	ND	ND	ND	ND
5		8.1	14.0	3.2	4,978	29.70	-	-	++	-	+	+
6		8.4	17.3	3.0		29.78	-	-	++	+	+	+
7		8.4	17.9	3.4		29.87	-	-	+	+	+	+
8	2.2	8.3	18.3	2.5	4,798	29.70	-	-	+	++	+	+
9	2.2	8.3	18.5	2.0		29.83	-	-	+	+	+	+
10	2.2	8.2	17.6	1.9		29.73	-	-	+	+	+	+
11	2.2	8.2	17.0	1.4		29.61	-	-	+	-	-	-
12		8.2	17.3	ND	5,817	29.72	-	-	-	-	+	+
13	2.2	8.1	18.0	ND		29.72	-	-	-	-	ND	ND
14		8.2	18.0	7.7	5,685	29.84	-	-	-	-	-	-
15		ND	ND	ND		ND	-	-	-	-	-	-
16		ND	ND	ND		ND	-	-	-	-	-	-
17		ND	ND	ND		ND	-	-	-	-	-	-
18		ND	ND	ND		ND	-	-	-	-	-	-
19		ND	ND	ND		ND	-	-	-	-	-	-
20	2.2	7.8	18.2	3.8		29.92	-	-	ND	ND	-	-
21		7.6	18.3	1.2	5,977	ND	-	-	-	-	-	-
22		ND	ND	ND	6,733	ND	-	-	-	-	-	-
23		7.1	18.8	2.0		ND	-	-	-	-	ND	ND
24		6.8	19.3	2.2		29.73	-	-	-	-	ND	ND
25		6.7	19.3	2.3		29.67	-	-	-	-	ND	ND
Mean		7.9	18.0	2.9		29.75						

X = Effluent; Y = Sludge
++ = motility

SURVIVAL AND DETECTION OF LEPTOSPIRES IN ANIMAL MANURE DISPOSAL

	Manure Environment					Survival (Rabbit Serum Agar)					
						Position X			Position Y		
Day	pH Mean	Ditch Temp. Mean (C)	D.O. Mean (ppm)	Total Solids (ppm)	Barom-eter Mean (in.)	Top	Middle	Bottom	Top	Middle	Bottom
Experiment No. 12 LS Studies of Seeded Ditch at Summer Temperatures											
0	5.9	21	4.5	8218	29.50	ND	ND	ND	ND	ND	ND
1	6.0	20	2.9	ND	29.80	+	+	-	+	-	-
2	6.0	21	1.7	ND	29.85	-	-	-	ND	-	-
3	6.3	19	1.0	ND	29.81	+	+	ND	+	-	+
4	6.3	21	1.3	ND	29.78	+	+	ND	-	-	+
5	6.4	22	8.9	8578	29.78	-	+	-	-	-	-
6	6.4	23	18.0	ND	29.78	-	+	-	+	-	-
7	6.4	20	17.0	ND	29.98	-	-	-	+	-	+
8	6.4	19	14.0	8373	29.76	-	-	-	+	-	+
9	6.4	17	11.0	ND	29.75	-	-	-	+	-	-
10	6.4	22	5.0	ND	29.61	-	+	+	-	-	-
11	6.5	23	4.0	ND	ND	ND	ND	ND	ND	ND	ND
12	6.7	24	3.0	7896	29.74	-	-	-	-	-	-
13	7.0	21	8.5	ND	29.75	-	-	-	-	-	-
14	6.9	19	14.2	ND	29.73	-	-	-	-	-	-
15	6.7	19	5.2	7334	29.75	-	-	-	-	-	-
16	6.3	20	1.0	ND	29.98	-	-	-	-	-	-
17	6.4	19	1.2	ND	29.71	-	-	-NG	-	-	-
18	6.4	19	2.0	ND	29.26	-	-	-NG	-	-	-
19	6.3	20	1.7	7226	29.15	-	-	-	-		
20	6.3	17	2.4	ND	29.44	+	-	+	+	-	-
21	6.4	20	2.2	ND	30.00	+	+	+	+	-	+
22	6.4	20	2.2	ND	31.50	+	+	+	+	+	+
23	6.4	19	2.1	ND	30.08	+	-	+	+	+	+

No. 12 LS

SURVIVAL AND DETECTION OF LEPTOSPIRES IN ANIMAL MANURE DISPOSAL

Manure Environment						Survival (Rabbit Serum Agar)					
						Position X			Position Y		
Day	pH Mean	Ditch Temp. Mean (C)	D.O. Mean (ppm)	Total Solids (ppm)	Barometer Mean (in.)	Top	Middle	Bottom	Top	Middle	Bottom
24	6.4	19	1.6	ND	ND	ND	ND	ND	ND	ND	ND
25	6.5	18	1.6	ND	29.75	ND	ND	ND	ND	ND	ND
26	6.6	20	0.8	ND	ND						
27-40	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
41	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
						+	+	+	+	+	+
										Contaminated	
42-46	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
47	ND	ND	ND	5713	ND	ND	ND	ND	ND	ND	ND
48	ND	ND	ND	5198	ND	+	+	+	+	+	+
49	7.8	16	4.4	ND	ND	+	+	+	+	+	+
50	7.8	17	4.0	ND	ND	+	+	+	+	+	+
51	7.8	23	4.8	ND	29.74	+	+	+	+	+	+
52	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
53	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
54	ND	ND	ND	4424	ND	-	+	+	+	+	+
55	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
56	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
57	ND	ND	ND	3990	ND	ND	ND	ND	ND	ND	ND
58	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
61	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
62	7.7	20	4.8	6237	29.74	+	+	+	+	+	+
63	7.8	24	5.0	ND	29.76	ND	ND	ND	ND	ND	ND
64	7.9	19	5.0	5160	29.82	+	+	+	+	+	+
65	7.9	18	5.3	ND	30.00	ND	ND	ND	ND	ND	ND

SURVIVAL AND DETECTION OF LEPTOSPIRES IN ANIMAL MANURE DISPOSAL

No. 12 LS

Manure Environment						Survival (Rabbit Serum Agar)					
						Position X			Position Y		
Day	pH Mean	Ditch Temp. Mean (C)	D.O. Mean (ppm)	Total Solids (ppm)	Barometer Mean (in.)	Top	Middle	Bottom	Top	Middle	Bottom
66	7.9	18	5.1	ND	ND	ND	ND	ND	ND	ND	ND
67	4.9	18	5.0	ND	29.70	ND	ND	ND	ND	ND	ND
68	7.9	18	4.5	5556	ND	+	+	+	+	+	+
69	7.9	17	4.4	ND	29.68	ND	ND	ND	ND	ND	ND
70	8.0	20	4.4	ND	29.67	-	-		+		
71	ND	ND	ND	4403	ND	ND	ND	ND	ND	ND	ND
72	ND	ND	ND	ND	ND	+	+	+	+	+	+
73	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
74	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
75	8.0	23	5.1	4229	29.80				read too soon		
76	8.0	23	6.0	ND	ND	ND	ND	ND	ND	ND	ND
77	8.0	21	6.3	ND	ND	+	+	+	+	+	+
78	8.0	24	6.6	8109	ND	ND	ND	ND	ND	ND	ND
79	8.0	20	6.0	ND	29.77				-	+	
80	8.2	20	5.6	ND	ND	ND	ND	ND	ND	ND	ND
81	8.2	23	6.0	ND	ND	ND	ND	ND	ND	ND	ND
82	8.0	22	6.0	8162	ND				+		
83	8.0	22	6.0	ND	29.78	ND	ND	ND	ND	ND	ND
84	8.0	21	5.8	ND	29.75				+		
85	8.1	21	5.8	ND	29.77	ND	ND	ND	ND	ND	ND
86	8.0	20	5.9	ND	29.78					+	
87	8.0	20	6.2	ND	29.69	ND	ND	ND	ND	ND	ND
88	7.9	22	7.0	ND	29.83	ND	ND	ND	ND	ND	ND
89	8.0	22	6.7	ND	29.92	ND	ND	ND	ND	ND	ND

No. 12 LS

SURVIVAL AND DETECTION OF LEPTOSPIRES IN ANIMAL MANURE DISPOSAL

Manure Environment						Survival (Rabbit Serum Agar)					
Day	pH Mean	Ditch Temp. Mean (C)	D.O. Mean (ppm)	Total Solids (ppm)	Barometer Mean (in.)	Position X			Position Y		
						Top	Middle	Bottom	Top	Middle	Bottom
90	8.0	22	6.2	ND	29.92	ND	ND	ND	ND	ND	ND
91	8.0	22	6.8	ND	30.00	ND	ND	ND	ND	ND	ND
92	8.0	22	7.0	ND	30.00	ND	ND	ND	ND	ND	ND
93	8.0	23	7.2	ND	30.04	ND	ND	ND	ND	ND	ND
94	8.0	24	6.5	ND	ND	ND	ND	ND	ND	ND	ND
95	8.0	25	6.2	ND	ND	ND	ND	ND	ND	ND	ND
96	8.0	25	5.8	ND	29.65	ND	ND	ND	ND	ND	ND
97	8.2	19	5.1	ND	ND	ND	ND	ND	ND	ND	ND
98	8.0	19	5.1	ND	27.86	ND	ND	ND	ND	ND	ND
99	8.0	18	6.3	ND	29.63	ND	ND	ND	ND	ND	ND
100	8.0	19	6.1	ND	29.61	ND	ND	ND	ND	ND	ND
101	7.9	18	5.9	ND	ND	ND	ND	ND	ND	ND	ND
102	8.0	19	6.9	ND	ND	ND	ND	ND	ND	ND	ND
103	8.1	20	6.8	ND	ND	ND	ND	ND	ND	ND	ND
104	8.0	19	6.6	ND	29.92	ND	ND	ND	ND	ND	ND
105	7.9	18	6.3	ND	29.76	ND	ND	ND	ND	N	ND
106	7.9	18	7.4	ND	29.78	ND	ND	ND	ND	ND	ND
107	8.0	19	8.0	ND	29.78	ND	ND	ND	ND	ND	ND
108	7.9	18	8.0	ND	ND	ND	ND	ND	ND	ND	ND
109	7.9	18	7.3	ND	29.81	ND	ND	ND	ND	ND	ND
110	7.8	19	7.0	ND	29.75	ND	ND	ND	ND	ND	ND
111	7.8	18	6.5	ND	29.75	ND	ND	ND	ND	ND	ND
112	7.8	18	6.9	ND	29.76	ND	ND	ND	ND	ND	ND
113	7.8	18	9.0	ND	29.76	ND	ND	ND	ND	ND	ND
114	7.9	19	8.5	ND	29.90	ND	ND	ND	ND	ND	ND
115	8.0	18	8.6	ND	ND	ND	ND	ND	ND	ND	ND
116	7.9	18	7.3	ND	ND	ND	ND	ND	ND	ND	ND

No. 12 LS

SURVIVAL AND DETECTION OF LEPTOSPIRES IN ANIMAL MANURE DISPOSAL

Manure Environment						Survival (Rabbit Serum Agar)					
						Position X			Position Y		
Day	pH Mean	Ditch Temp. Mean (C)	D.O. Mean (ppm)	Total Solids (ppm)	Barometer Mean (in.)	Top	Middle	Bottom	Top	Middle	Bottom
117	7.8	18	9.1	ND	29.93	ND	ND	ND	ND	ND	ND
118	8.0	18	11.0	ND	29.94	ND	ND	ND	ND	ND	ND
119	8.1	18	8.8	ND	29.88	ND	ND	ND	ND	ND	ND
120	8.0	19	10.2	ND	29.76	ND	ND	ND	ND	ND	ND
121	8.0	18	10.5	ND	29.52	ND	ND	ND	ND	ND	ND
122	8.0	17	11.0	ND	29.91	ND	ND	ND	ND	ND	ND
123	8.2	17	10.6	ND	29.61	ND	ND	ND	ND	ND	ND
124	8.0	17	9.3	ND	29.65	ND	ND	ND	ND	ND	ND
125	7.9	17	10.8	ND	29.78	ND	ND	ND	ND	ND	ND
126					PEN NOT WORKING						
127	7.9	17	10.0	ND	29.78	ND	ND	ND	ND	ND	ND
128	8.0	16	11.0	ND	29.76	ND	ND	ND	ND	ND	ND
129	8.0	17	10.0	ND	29.80	ND	ND	ND	ND	ND	ND
130	8.0	17	14.0	ND	29.75	ND	ND	ND	ND	ND	ND
131	7.9	18	10.7	ND	ND	ND	ND	ND	ND	ND	ND
132	8.0	17	14.0	ND	29.75	ND	ND	ND	ND	ND	ND
133	8.0	16	14.0	ND	29.76	ND	ND	ND	ND	ND	ND
134	8.0	18	11.8	ND	29.26	ND	ND	ND	ND	ND	ND
135	8.0	18	11.0	ND	ND	ND	ND	ND	ND	ND	ND
136	8.0	18	11.0	ND	ND	ND	ND	ND	ND	ND	ND
137	8.0	18	11.0	ND	ND	ND	ND	ND	ND	ND	ND
138	8.0	19	11.0	ND	29.79	ND	ND	ND	ND	ND	ND
139	8.0	20	12.0	ND	29.92	ND	ND	ND	ND	ND	ND

No. 12 LS

SURVIVAL AND DETECTION OF LEPTOSPIRES IN ANIMAL MANURE DISPOSAL

	Manure Environment					Survival (Rabbit Serum Agar)					
						Position X			Position Y		
Day	pH Mean	Ditch Temp. Mean (C)	D.O. Mean (ppm)	Total Solids (ppm)	Barometer Mean (in.)	Top	Middle	Bottom	Top	Middle	Bottom
140	8.0	20	12.0	ND	30.18	ND	ND	ND	ND	ND	ND
141					STOPPED						
142	7.8	20	12.0	ND	30.19	+	+	+	+	+	+*
143	7.8	21	12.0	ND	ND	ND	ND	ND	ND	ND	ND
144	8.0	21	12.0	ND	ND	ND	ND	ND	ND	ND	ND
145				END	OF EXPERIMENT						
Means	7.6	19.6	7.1	6367	30.15						

* Isolated + D.F.

ENGINEERING STUDIES OF OXIDATION DITCH OPERATION

Appendix - B

Velocity Distribution Studies

Table 2B

Test Number 1

Rotor Immersion Depth 1/2"

Water Depth 2"

Rotor Position A

	Lateral Position		
Station	I	II	III
	Velocity*		
1	.04	.12	.07
2	.07	.14	.12
3	.07	.19	.16
4	.10	.21	.04
5	.10	.25	.14
6	0	.39	0
7	.01	.42	.04
8	0	.39	.27
9	.16	.25	.14
10	.14	.19	.14
11	.30	19	.30
12	.27	.14	.04
13	.07	.10	.19
14	.07	.19	.16
15	0	.16	.16
16	0	.14	.25
17	0	.16	.30
18	0	.14	.30
19	-.01	.16	.36
20	0	.30	.25
21	.04	.19	.07
22	.30	.14	.01
23	.19	.14	.01
24	.25	.14	.07

Table 3B

Test Number 2

Rotor Immersion Depth 1/2"

Water Depth 2"

Rotor Position B

	Lateral Position		
Station	I	II	III
	Velocity*		
1	0	.10	.30
2	0	.12	.27
3	0	.12	.21
4	0	.10	.21
5	.01	.12	.16
6	.01	.07	.16
7	.01	.12	.12
8	.21	.14	.10
9	.25	.16	0
10	.30	.16	.01
11	.2	.27	.10
12	0	.30	.25
13	.16	.16	.10
14	0	.30	0
15	.04	.19	.04
16	.07	.16	.10
17	.07	.12	.10
18	.07	.12	.07
19	.12	.10	.07
20	.19	.12	.04
21	.21	.10	0
22	.36	.14	0
23	.04	.27	.04
24	0	.36	.30

*Velocities measured in units of feet per second.

Velocity Distribution Studies (con't)

Table 4B

Test Number 3

Rotor Immersion Depth 1/2"

Water Depth 2"

Rotor Position E

	Lateral Position		
Station	I	II	III
	Velocity*		
1	-.01	.48	0
2	.33	.27	.01
3	.33	.33	-.09
4	.21	.25	.12
5	.14	.27	.14
6	.10	.21	.19
7	.16	.21	.12
8	.39	.16	.10
9	.36	.19	.04
10	.42	.21	.04
11	.21	.39	.19
12	0	.39	.46
13	0	.19	.39
14	0	.25	.42
15	.01	.27	.36
16	.07	.21	.36
17	.04	.25	.33
18	.07	.25	.25
19	.10	.16	.19
20	.36	.25	.07
21	.30	.13	.04
22	.36	.16	.04
23	.16	.30	.12
24	---	---	.33

Table 5B

Test Number 4

Rotor Immersion Depth 1"

Water Depth 2"

Rotor Position A

	Lateral Position		
Station	I	II	III
	Velocity*		
1	0	.52	.52
2	.48	.52	-.01
3	.27	.36	.19
4	.25	.33	.16
5	.27	.30	.14
6	.21	.27	.16
7	.25	.27	.19
8	.52	.30	.14
9	.48	.27	.10
10	.48	.33	.12
11	.45	.42	.27
12	0	.45	.48
13	0	.30	.48
14	0	.27	.48
15	.01	.21	.30
16	.01	.27	.39
17	.04	.25	.27
18	.07	.27	.27
19	.07	.27	.27
20	.42	.27	.16
21	.48	.30	.12
22	.45	.27	.07
23	.21	.39	.25
24	---	---	---

* Velocities measured in units of feet per second

Velocity Distribution Studies (con't)

Table 6B

Test Number 5

Rotor Immersion Depth 1"

Water Depth 2"

Rotor Position B

	Lateral Position		
Station	I	II	III
	Velocity*		
1	.01	.25	.42
2	-.07	.25	.42
3	.01	.25	.39
4	.01	.30	.42
5	.16	.30	.36
6	.07	.27	.33
7	.12	.25	.30
8	.48	.42	.14
9	.42	.33	.10
10	.48	.36	.07
11	.33	.36	.25
12	.33	.52	.52
13	0	.52	-.33
14	.46	.48	-.16
15	.46	.48	-.21
16	.48	.48	-.16
17	.33	.42	.30
18	.33	.42	.30
19	.36	.39	.33
20	.48	.36	.19
21	.48	.39	.19
22	.48	.36	.12
23	.21	.46	.27
24	0	.48	.48

Table 7B

Test Number 6

Rotor Immersion Depth 1"

Water Depth 2"

Rotor Position E

	Lateral Position		
Station	I	II	III
	Velocity*		
1	.21	.27	.14
2	.21	.27	.12
3	.19	.33	.14
4	.33	.36	.07
5	.39	.39	0
6	.42	.42	-.10
7	0	.46	-.16
8	.16	.46	.42
9	.46	.39	.21
10	.52	.33	.07
11	.48	.30	.01
12	.46	.33	.10
13	.12	.33	.25
14	.12	.33	.25
15	.01	.30	.33
16	0	.27	.36
17	0	.36	.36
18	.07	.30	.48
19	.10	.36	.48
20	0	.42	.48
21	.30	.39	.25
22	.42	.21	.10
23	.42	.19	.04
24	.39	.27	.12

*Velocities measured in units of feet per second.

Velocity Distribution Studies (con't)

Table 8B

Test Number 7

Rotor Immersion Depth 1½"

Water Depth 2"

Rotor Position A

Station	Lateral Position I	Lateral Position II	Lateral Position III
	Velocity*		
1	.36	.36	.36
2	.36	.36	.36
3	.30	.36	.30
4	.30	.36	.30
5	.33	.36	.25
6	.30	.39	.25
7	.27	.46	.07
8	---	---	---
9	.25	.36	.30
10	.42	.36	.14
11	.46	.30	.04
12	.42	.36	.25
13	.16	.36	.39
14	.12	.36	.42
15	.33	.39	.42
16	.04	.36	.42
17	0	.42	.48
18	.27	.39	.48
19	.07	.42	.48
20	.12	.48	.48
21	.36	.42	.30
22	.48	.30	.12
23	.46	.30	.04
24	.42	.36	.25

Table 9B

Test Number 8

Rotor Immersion Depth 1½"

Water Depth 2"

Rotor Position B

Station	Lateral Position I	Lateral Position II	Lateral Position III
	Velocity*		
1	0	.33	.48
2	0	.36	.48
3	0	.33	.42
4	.01	.33	.42
5	.10	.36	.42
6	.14	.33	.39
7	.12	.36	.42
8	.42	.36	.19
9	.36	.33	.07
10	.48	.36	.14
11	.07	.42	.27
12	---	---	---
13	.25	.45	0
14	.46	.46	0
15	.39	.39	.04
16	.42	.36	.19
17	.36	.36	.21
18	.33	.36	.16
19	.33	.36	.27
20	.45	.36	.12
21	.46	.30	.07
22	.48	.36	.12
23	.07	.42	.27
24	.01	.39	.48

*Velocities measured in units of feet per second.

Velocity Distribution Studies (con't)

Table 10B

Test Number 9

Rotor Immersion Depth 1½"

Water Depth 2"

Rotor Position E

Lateral Position

Station	I	II	III
	Velocity*		
1	.14	.33	.16
2	.21	.42	.21
3	.21	.33	.27
4	.30	.36	.30
5	.33	.33	.30
6	.27	.30	.30
7	.25	.30	.25
8	.42	.25	.19
9	.42	.25	.12
10	.42	.27	.10
11	.12	.42	.25
12	.01	.42	.42
13	-.12	.27	.48
14	0	.33	.42
15	.07	.36	.36
16	.21	.33	.36
17	.25	.36	.36
18	.27	.33	.30
19	.30	.36	.36
20	---	---	---
21	.42	.27	.07
22	.42	.36	.12
23	.36	.42	.30
24	---	---	---

Table 11B

Test Number 10

Rotor Immersion Depth ½"

Water Depth 3"

Rotor Position A

Lateral Position

Station	I	II	III
	Velocity*		
1	.12	.19	.14
2	.16	.19	.12
3	.16	.16	.10
4	.14	.19	.10
5	.10	.27	.04
6	.16	.30	-.07
7	0	.46	0
8	-.07	---	---
9	.12	.30	.10
10	.36	.19	.04
11	.25	.14	.04
12	.25	.19	.10
13	.14	.19	.14
14	0	.16	.14
15	.04	.19	.25
16	.04	.16	.27
17	0	.14	.33
18	-0.1	.14	.27
19	-0.1	.16	.42
20	0	.27	.30
21	.12	.25	.14
22	.30	.14	.07
23	.25	.14	.07
24	.30	.14	.07

*Velocities measured in units of feet per second.

Velocity Distribution Studies (con't)

Table 12B

Test Number 11

Rotor Immersion Depth ½"

Water Depth 3"

Rotor Position B

Station	Lateral Position		
	I	II	III
	Velocity*		
1	0	.12	.36
2	0	.14	.36
3	0	.14	.36
4	0	.19	.30
5	.01	.21	.19
6	.10	.16	.19
7	.07	.21	.21
8	.36	.25	.12
9	.42	.19	.07
10	.42	.21	.12
11	.12	.33	.21
12	---	---	---
13	.12	.48	-.07
14	.12	.39	-.07
15	.10	.33	.12
16	.21	.25	.07
17	.21	.21	.10
18	.14	.16	.16
19	.19	.16	.14
20	.14	.16	.10
21	.14	.14	.10
22	.36	.16	.07
23	0	.30	.12
24	0	.25	.27

Table 13B

Test Number 12

Rotor Immersion Depth ½"

Water Depth 3"

Rotor Position E

Station	Lateral Position		
	I	II	III
	Velocity*		
1	-.07	.48	0
2	.12	.39	-.07
3	.21	.30	.01
4	.14	.25	.16
5	.21	.25	.16
6	.16	.25	.19
7	.14	.19	.19
8	.36	.19	.07
9	.36	.14	.04
10	.42	.25	.10
11	.01	.36	.21
12	0	.33	.33
13	-.01	.21	.36
14	0	.19	.36
15	.01	.25	.33
16	.10	.21	.27
17	.12	.21	.19
18	.10	.25	.25
19	.14	.25	.21
20	.30	.25	.14
21	.36	.19	.10
22	.36	.19	.10
23	.10	.30	.16
24	---	---	---

*Velocities measured in units of feet per second.

Velocity Distribution Studies (con't)

Table 14B

Test Number 13

Rotor Immersion Depth 1"

Water Depth 3"

Rotor Position A

Station	I	II	III
	Velocity*		
1	.25	.33	.25
2	.27	.30	.16
3	.27	.33	.25
4	.33	.30	.16
5	.33	.36	.12
6	.30	.39	-.16
7	.36	.48	0
8	---	---	---
9	-.07	.42	.30
10	.48	.27	.16
11	.42	.25	.12
12	.42	.30	.12
13	.14	.36	.27
14	.16	.33	.27
15	.19	.33	.36
16	.12	.30	.39
17	0	.33	.46
18	0	.21	.46
19	-.07	.25	.48
20	0	.46	.46
21	.25	.33	.30
22	.48	.21	.14
23	.48	.25	.10
24	.46	.30	.07

Table 15B

Test Number 14

Rotor Immersion Depth 1"

Water Depth 3"

Rotor Position B

Station	I	II	III
	Velocity*		
1	.07	.19	.46
2	.12	.30	.42
3	.07	.27	.42
4	.12	.30	.42
5	.16	.33	.39
6	.16	.30	.33
7	.21	.33	.36
8	.42	.33	.16
9	.48	.30	.12
10	.48	.33	.16
11	.33	.42	.39
12	---	---	---
13	.10	.48	-.16
14	.30	.48	-.07
15	.30	.36	.07
16	.21	.33	.14
17	.19	.33	.30
18	.19	.27	.22
19	.21	.30	.22
20	.42	.30	.16
21	.42	.25	.04
22	.48	.30	.14
23	.01	.42	.22
24	.01	.27	.42

*Velocities measured in units of feet per second.

Velocity Distribution Studies (con't)

Table 16B

Test Number 15

Rotor Immersion Depth 1"

Water Depth 3"

Rotor Position E

	Lateral Position		
Station	I	II	III
	Velocity*		
1	-.12	.48	.07
2	-.01	.39	.21
3	.16	.39	.30
4	.25	.30	.33
5	.19	.27	.30
6	.27	.33	.27
7	.25	.30	.27
8	.42	.30	.14
9	.48	.25	.07
10	.48	.30	.16
11	.07	.48	.30
12	.04	.39	.42
13	-.07	.10	.48
14	.16	.10	.46
15	0	.30	.39
16	.0	.36	.36
17	.21.	.33	.30
18	.19	.27	.33
19	.16	.33	.27
20	.14	.33	42
21	.42	.25	.07
22	.48	.30	.12
23	.36	.48	.21
24	---	---	---

Table 17B

Test Number 16

Rotor Immersion Depth 1½"

Water Depth 3"

Rotor Position A

	Lateral Position		
Station	I	II	III
	Velocity*		
1	.33	.36	.21
2	.36	.33	.21
3	.39	.33	.14
4	.42	.33	.16
5	.46	.33	.07
	.46	.30	.12
7	.46	.48	.01
8	---	---	---
9	.39	.42	.33
10	.48	.36	.21
11	.48	.30	.16
12	.48	.33	.16
13	.33	.33	.33
14	.25	.36	.36
15	.9	.27	.27
16	.16	.33	.39
17	.07	.36	.48
18	-.07	.21	.50
19	-.16	.27	.48
20	.07	.46	.48
21	.16	.42	.33
22	.48	.25	.14
23	.48	.21	.07
24	.48	.30	.07

*Velocities measured in units of feet per second.

Velocity Distribution Studies (con't)

Table 18B

Test Number 17

Rotor Immersion Depth 1½"

Water Depth 3"

Rotor Position B

	Lateral Position		
Station	I	II	III
		Velocity*	
1	0	.16	.48
2	0	.25	.48
3	.04	.30	.42
4	.12	.36	.39
5	.14	.30	.42
6	.21	.36	.42
7	.30	.36	.30
8	.48	.36	.14
9	.48	.33	.10
10	.48	.36	.19
11	.48	.48	.36
12	---	---	---
13	.48	.50	.12
14	.50	.33	.12
15	.46	.36	.07
16	.42	.36	.14
17	.42	.30	.14
18	.42	.36	.14
19	.36	.33	.25
20	.48	.33	.12
21	.48	.27	.12
22	.42	.27	.16
23	.12	.42	.33
24	0	.33	.42

Table 19B

Test Number 18

Rotor Immersion Depth 1½"

Water Depth 3"

Rotor Position E

	Lateral Position		
Station	I	II	III
		Velocity*	
1	.21	.50	0
2	.30	.39	.07
3	.36	.27	.07
4	.36	.33	.16
5	.33	33	.19
6	.36	.36	.27
7	.36	.36	.27
8	.48	.30	.21
9	.48	.30	.12
10	.42	.36	.19
11	.16	.42	.33
12	.04	.30	.42
13	-.16	.30	.48
14	.07	.27	.42
15	.12	.36	.42
16	.16	.39	.48
17	.27	.33	.30
18	.19	.36	.33
19	.30	.36	.27
20	.48	.36	.19
21	.42	.27	.07
22	.42	.33	.14
23	.36	.42	.25
24	---	---	---

*Velocities measured in units of feet per second.

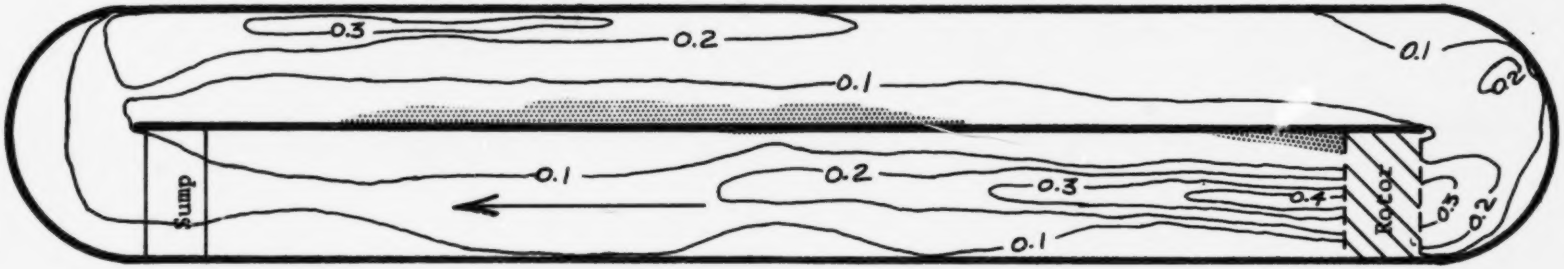


Figure 13

Velocity Distribution Test No. 1
Water Depth - 2"
Rotor Immersion Depth - 1/2"

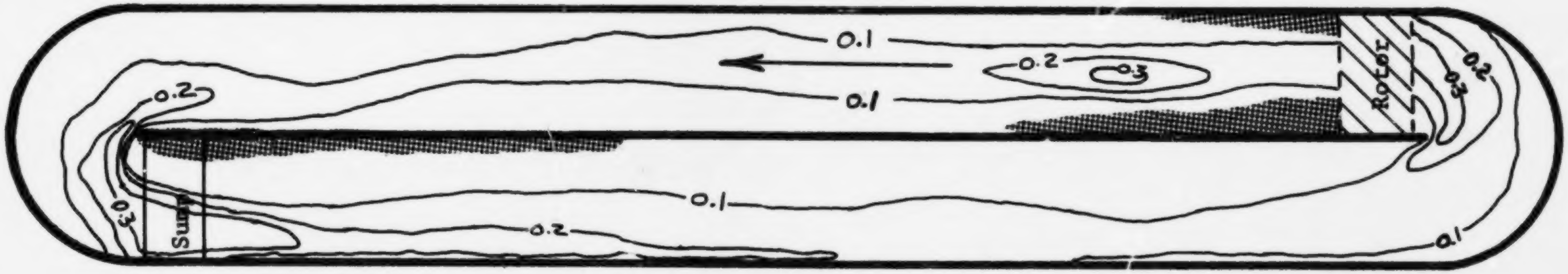


Figure 14

Velocity Distribution Test No. 2
Water Depth - 2"
Rotor Immersion Depth - 1/2"

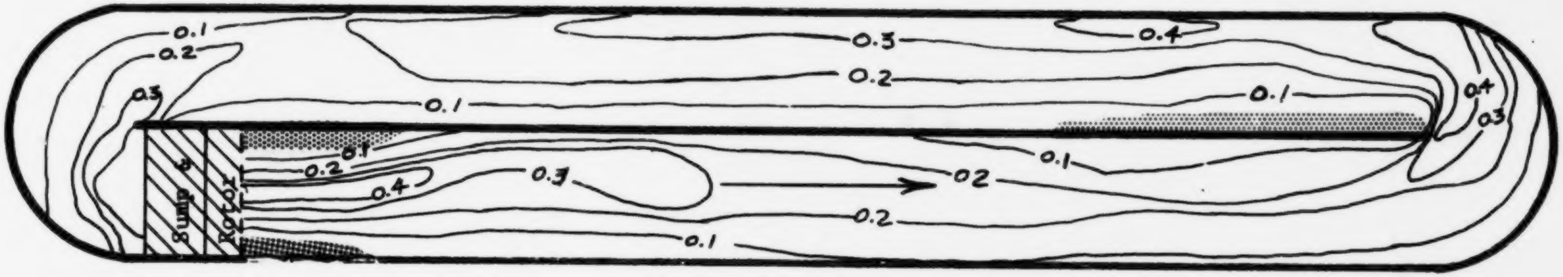


Figure 15

Velocity Distribution Test No. 3
Water Depth - 2"
Rotor Immersion Depth - 1/2"

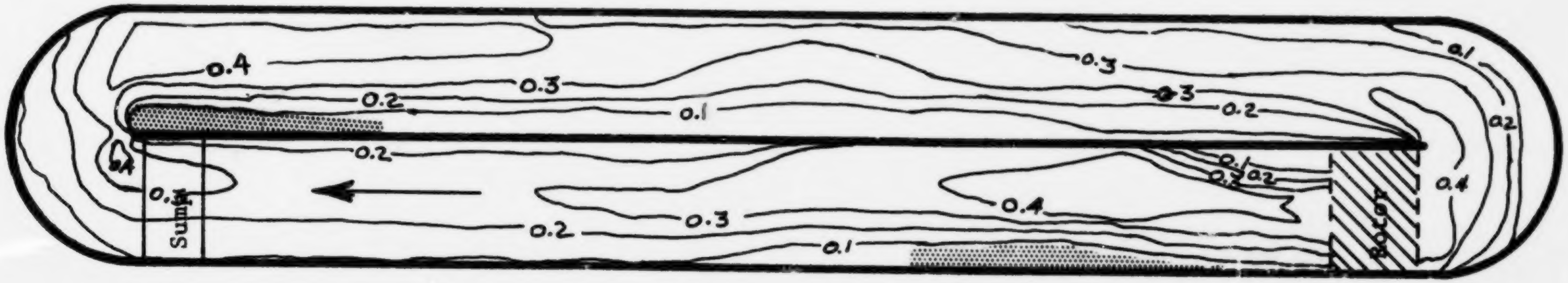


Figure 16

Velocity Distribution Test No. 4
Water Depth - 2"
Rotor Immersion Depth - 1"

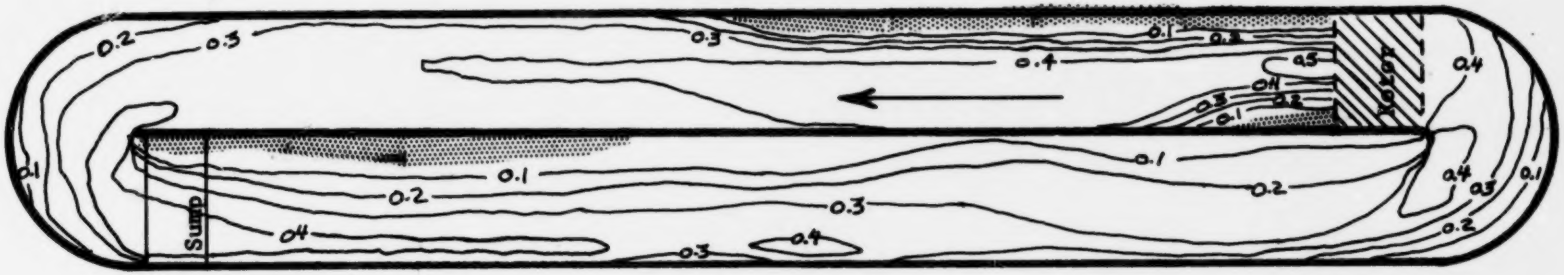


Figure 17

Velocity Distribution Test No. 5
Water Depth - 2"
Rotor Immersion Depth - 1"

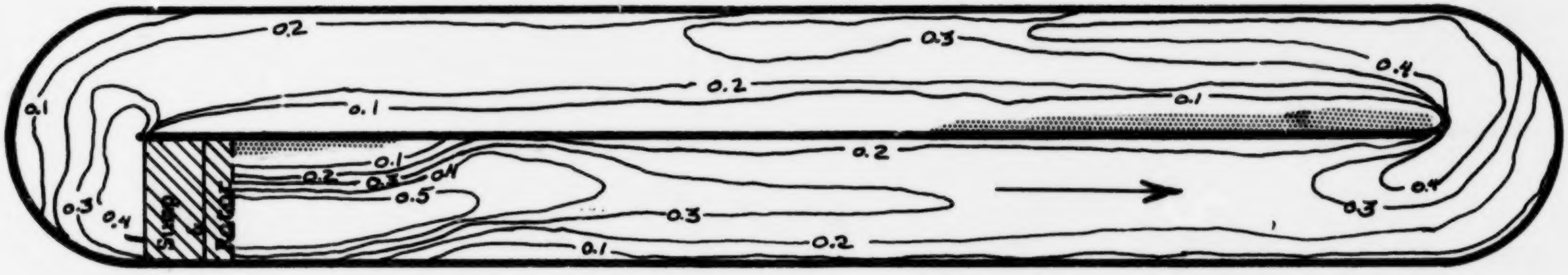


Figure 18

Velocity Distribution Test No. 6
Water Depth - 2"
Rotor Immersion Depth - 1"

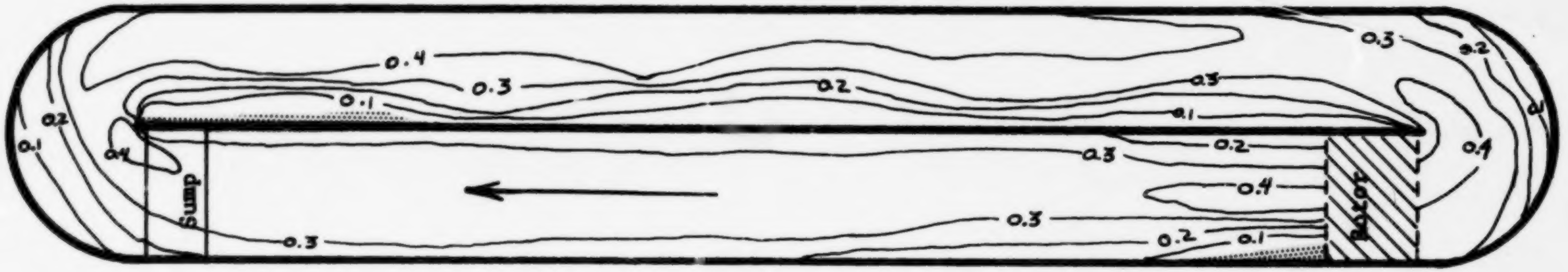


Figure 19

Velocity Distribution Test No. 7
Water Depth - 2"
Rotor Immersion Depth - 1 1/2"

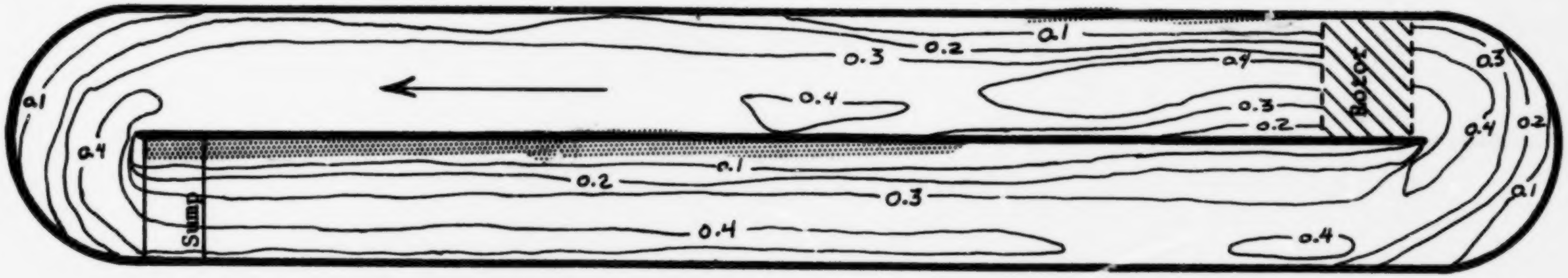
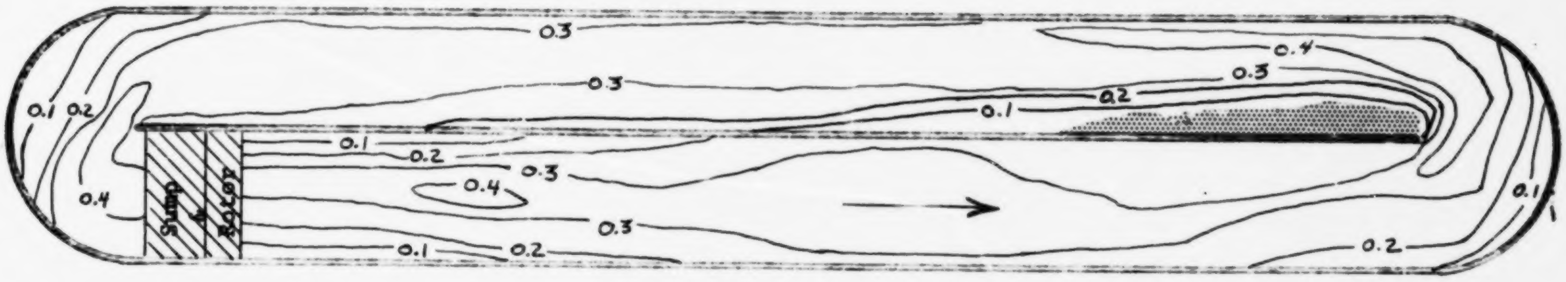


Figure 20

Velocity Distribution Test No. 8
Water Depth - 2"
Rotor Immersion Depth - 1 1/2"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0.4
0.3
0.3
0.1
0.2
0.1
0.2
0.1
0.2
0.3
0.4
0.4
Sump
Rotor
0.3
0.1
0.2
0.2
0.1

Figure 21
Velocity Distribution Test No. 9
Water Depth - 2"
Rotor Immersion Depth - 1 1/2"

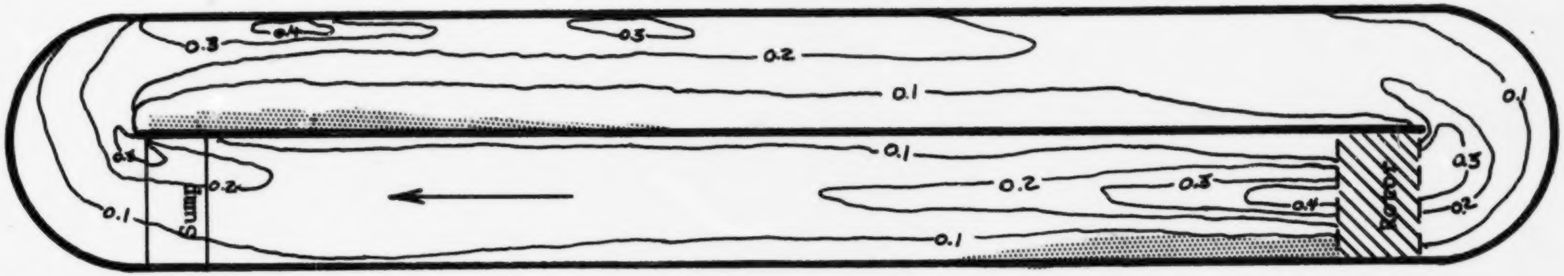

0.3
0.4
0.3
0.2
0.1
0.1
0.3
0.2
0.2
0.3
0.4
0.1
Rotor
0.3
0.2
0.1
0.1
Sump

Figure 22
Velocity Distribution Test No. 10
Water Depth - 3"
Rotor Immersion Depth - 1/2"

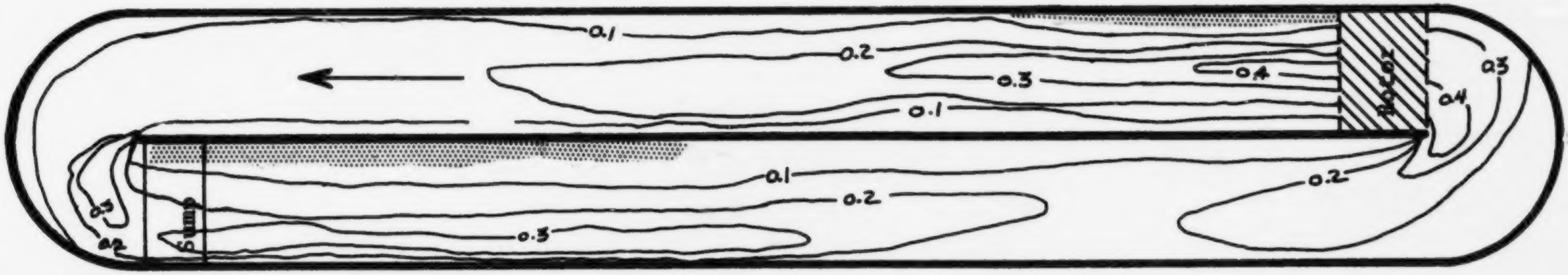

0.1
0.2
0.3
0.4
0.1
Rotor
0.4
0.3
0.1
0.2
0.2
0.3
0.3
0.2
Sump

Figure 23
Velocity Distribution Test No. 11
Water Depth - 3"
Rotor Immersion Depth - 1/2"

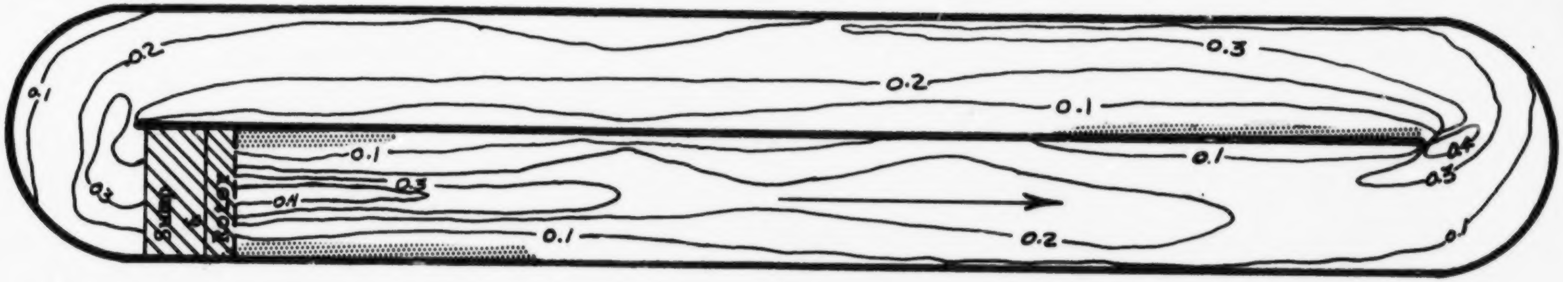

0.1
0.2
0.3
Sump
Rotor
0.1
0.3
0.4
0.1
0.2
0.1
0.3
0.2
0.1
0.1
0.4
0.3
0.2
0.1

Figure 24
Velocity Distribution Test No. 12
Water Depth - 3"
Rotor Immersion Depth - 1/2"

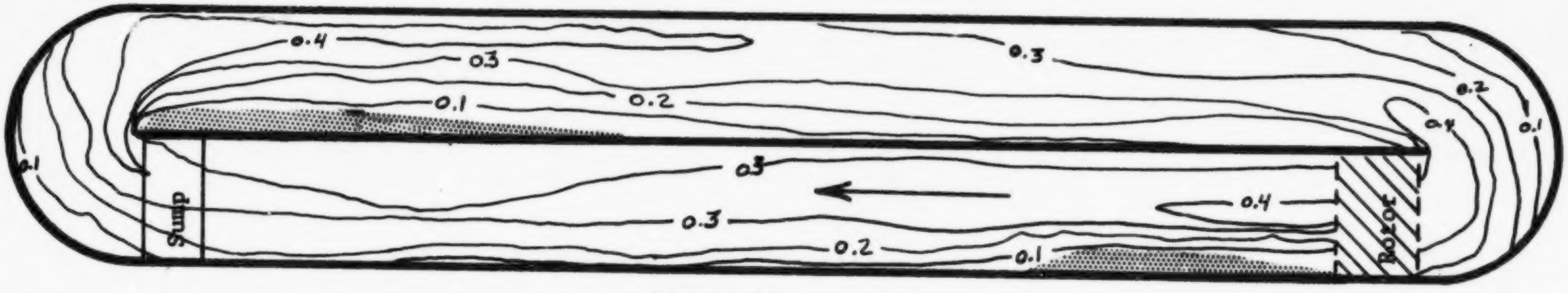

0.4
0.3
0.1
0.2
0.3
0.1
Sump
0.3
0.3
0.2
0.1
0.4
Rotor
0.2
0.4
0.1

Figure 25
Velocity Distribution Test. No. 13
Water Depth - 3"
Rotor Immersion Depth - 1"

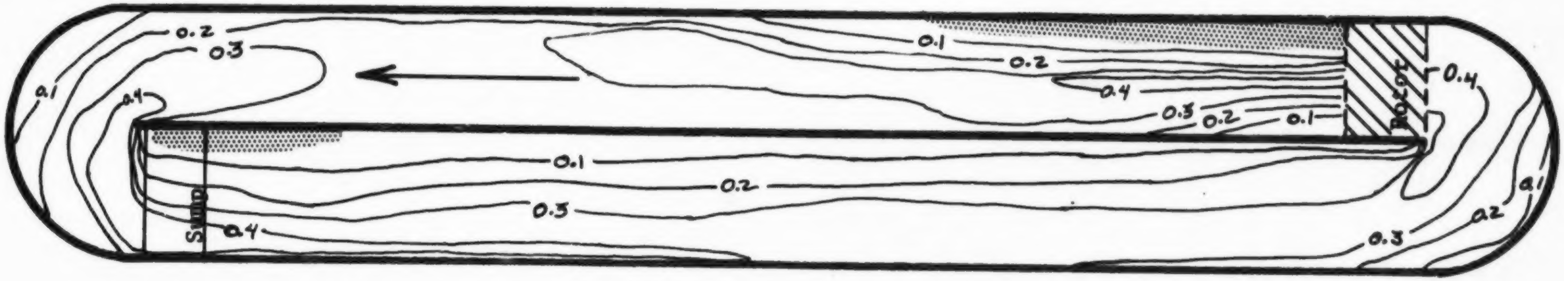


Figure 26

Velocity Distribution Test. No. 14
Water Depth - 3"
Rotor Immersion Depth - 1"

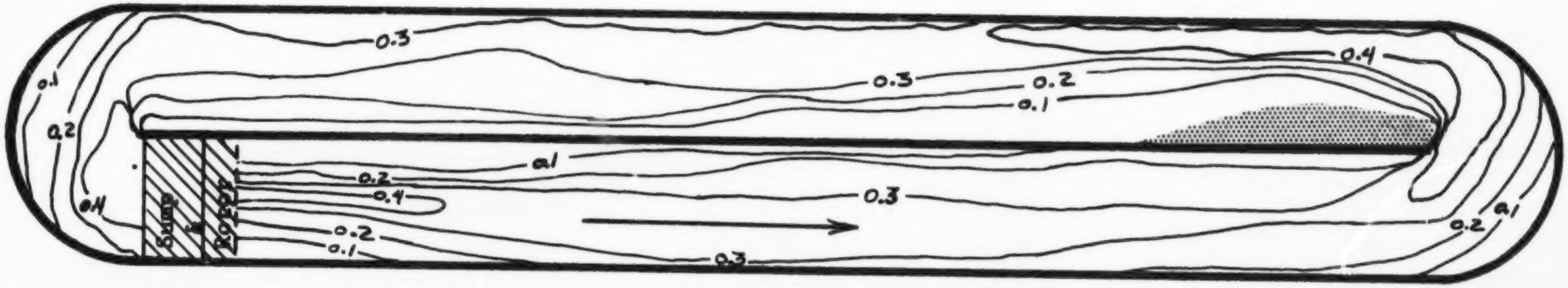
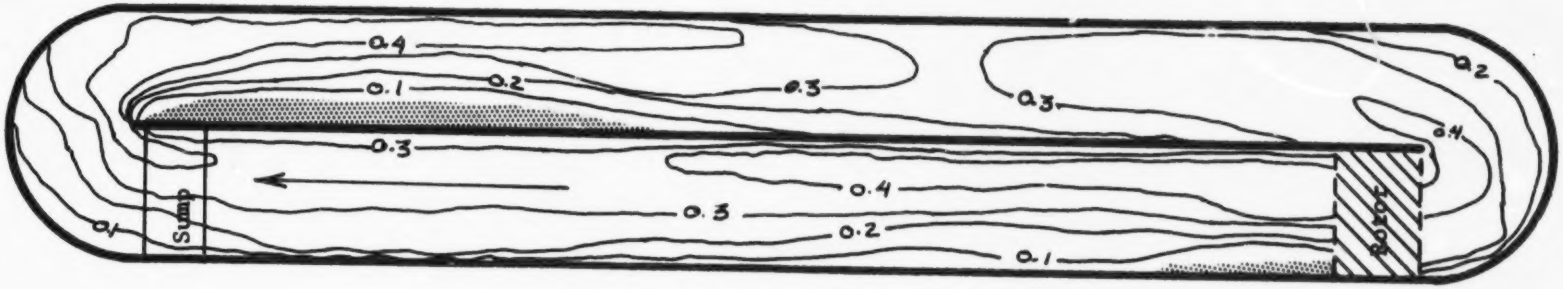


Figure 27

Velocity Distribution Test No. 15
Water Depth - 3"
Rotor Immersion Depth - 1"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0.1
0.2
0.3
0.3
0.2
0.4
0.3
0.4
0.3
0.2
0.1
0.1
Sump
Rotor

Figure 28
Velocity Distribution Test No. 16
Water Depth - 3"
Rotor Immersion Depth - 1 1/2"

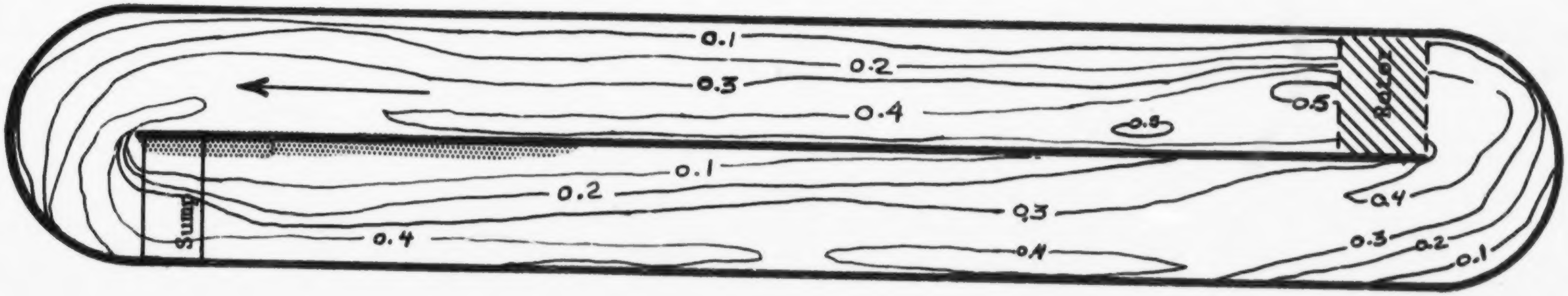
0.1
0.2
0.3
0.4
0.5
0.5
0.1
0.2
0.3
0.4
0.4
0.4
0.3
0.2
0.1
Sump
Rotor

Figure 29
Velocity Distribution Test No. 17
Water Depth - 3"
Rotor Immersion Depth - 1 1/2"

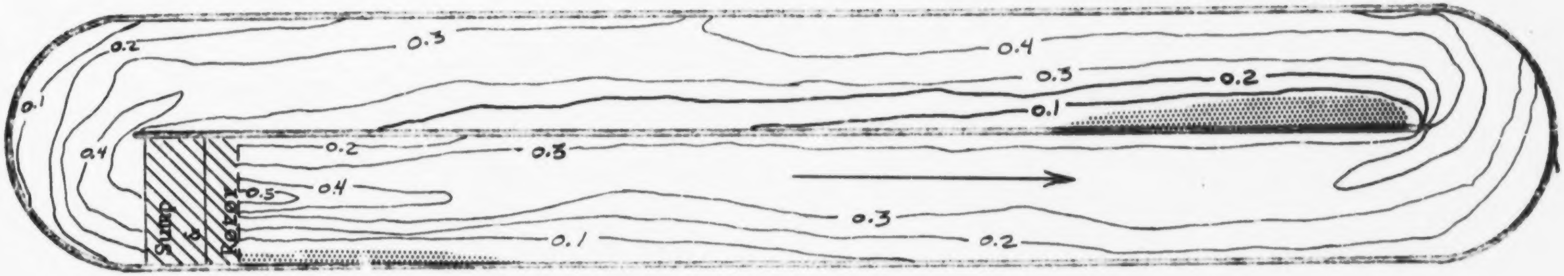

Figure 30
Velocity Distribution Test No. 18
Water Depth - 3"
Rotor Immersion Depth - 1 1/2"